1. General Information

ID 12002-85-3

Date December 22, 2007

1.0 SUBSTANCE INFORMATION

Generic Name	:	Zinc naphthenate
Chemical Name	:	Naphthenic acids, zinc salts
CAS Registry No.	:	12001-85-3
Component CAS Nos.	:	
EINECS No.	:	
Structural Formula	:	$Zn(MRCO_2)(NRCO_2)$ Where, R = alkyl group with a chain length of 0 to 10 carbon atoms, M & N are typically one or two fused rings (usually cyclopentane but occasionally cyclohexane or heptane rings) that may contain one or more alkyl substitutions. The total number of carbon atoms in M or N ranges from about 9 to 25. In some cases, no fused ring is present and M or N may be straight-chain or multiple branched carbon/hydrogen/oxygen molecules.
Additional description	:	This compound is the reaction product of zinc oxide and naphthenic acids, a petroleum refining by-product. Depending on the source of naphthenic acid, this compound may also contain 5 –20% paraffinic hydrocarbons which have a similar distillation range to the carboxylic acids. They cannot be removed by standard chemical processing and are not considered to be impurities, but rather legitimate components of naphthenic acid. Zinc naphthenate may be a viscous liquid containing 8-10% zinc or a solid containing 16% zinc (EPA, 1992).
Molecular Weight	:	Ranges from approximately 381 to 813
Synonyms and Tradenames	:	Fungitrol
References	:	EPA (1992). Drinking water toxicity profiles. U.S. Environmental Protection Agency. Report prepared for Army Medical Research and Development Command, Fort Detrick, Maryland. NTIS No. PB93122406. [Subsequently referenced as EPA, (1992)] EPA (1981). Chemical Hazard Information Profile - Draft Report. Cobalt Naphthenate, CAS No. 61789-51-3. U.S. Environmental Protection Agency, Office of Toxic Substances. 8 p. [Subsequently referenced as EPA, (1981)]

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	°C
Decomposition	:	at °C
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** The pure form of zinc naphthenate is a cold flowing solid at room temperature. Because this substance is a mixture of many of different compounds, a distinct melting point is not expected. The melting point is the result of the transition from a highly ordered crystal form of a compound to the disordered liquid form. Zinc naphthenate is not expected to have a distinct melting point because it is highly disordered as a solid due to its unique chemical composition.
Reliability	:	
Reference	:	

2.2 BOILING POINT

Type	:	
Guideline/method	:	ASTM D86-82
Value	:	116°C initial boiling point (pressure not specified)
Decomposition	:	Yes at 255°C
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (purity = 97%; 14.3% Zn)
Method	:	
Method detail	:	
Result	:	
Remark	:	Test material was a very viscous liquid (i.e., light brown paste)
Reliability	:	(1) Reliable without restrictions.
Reference	:	Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P.

2.3 DENSITY

Type	:	
Guideline/method	:	ASTM D1475-60 (reapproved 1980)
Value	:	1.118 g/ml at 20°C
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (purity = 97%; 14.3% Zn)

Method	:	
Method detail	:	
Result	:	
Remark	:	Test material was a very viscous liquid (i.e., light brown paste)
Reliability	:	(1) Reliable without restrictions.
Reference		Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P.

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	<0.1 mm Hg (temperature not specified)
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	Mixture of 84% zinc naphthenate (14.5% Zn) and 16% petroleum hydrocarbon oil (CAS No. 64742-52-5)
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	Product MSDS, Sheperd Chemical Co.

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	1.10 at 20 °C
pH value	:	
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (purity = 97%; 14.7% Zn)
Method	:	
Method detail	:	Zinc as metal content in octanol was measured using ASTM method D2373-85. Zinc in water was measured by atomic absorption spectroscopy according to ASTM method E885-88.
Result	:	
Remark	:	
Reliability	:	(2) Reliable with restrictions. Test was not conducted at different pH values or in buffered water.
Reference	:	Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P.

2.6.1 SOLUBILITY IN WATER

Type	:	
Guideline/method	:	Water Solubility ASTM Method D2373-85
Value	:	80 mg/L at 20°C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	

PKa	:	at °C
Description	:	
Stable	:	
Deg. product	:	
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (purity = 97%; 14.7% Zn)
Deg. products CAS#	:	
Method	:	Flask Method conducted in accordance with reference (3) of Guideline 63-8(d) 40 CFR Part 158.
Method detail	:	Zinc in water was measured by atomic absorption spectroscopy according to ASTM method E885-88
Result	:	
Remark	:	Test material was a very viscous liquid (i.e., light brown paste)
Reliability	:	(1) Reliable without restrictions.
Reference	:	Grove, S.L. 1990. Technical grade zinc naphthenate – product chemistry physical and chemical characteristics. Mooney Chemicals, Inc. Laboratory. Laboratory project Identification number F-24044-P

2.7 FLASH POINT

Type	:	
Guideline/method	:	
Value	:	>200 °C
Year	:	
GLP	:	
Test substance	:	Mixture of 84% zinc naphthenate (14.5% Zn) and 16% petroleum hydrocarbon oil (CAS No. 64742-52-5)
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	Product MSDS, Sheperd Chemical Co.

3.1.1 PHOTODEGRADATION

Type				
Guideline/method	:			
Light source	:			
Light spectrum	:			
Relative intensity	:	based on		
Spectrum of substance	:	lambda (max, >295nm)	:	
		epsilon (max)	:	
		epsilon (295)	:	
Conc. of substance	:		at	°C
DIRECT PHOTOLYSIS				
Halflife (t1/2)	:			
Degradation	:	% after		
Quantum yield	:			
INDIRECT PHOTOLYSIS				
Sensitizer	:			
Conc. of sensitizer	:			
Rate constant	:			
Degradation	:			
Deg. product	:			
Year	:			
GLP	:			
Test substance	:			
Deg. products CAS#	:			
Method	:			
Method detail	:			
Result	:			
Remark	:			
Reliability	:			
Reference	:			

3.1.2 DISSOCIATION

Type	:	Dissociation constant determination
Guideline/method	:	OECD 112
pKa	:	7.31 and 9.18 at 20°C
Year	:	2002
GLP	:	Yes
Test substance	:	Zinc naphthenate (54458-2), lot number 20131MI, received from Aldrich Chemical Company. Clear gold liquid, purity not reported.
Approx. water solubility	:	500 mg/L as determined visually in preliminary study
Method	:	OECD Guideline 112, Dissociation Constants in Water
Method detail	:	Three replicate samples of zinc naphthenate were prepared at a nominal concentration of 250 mg/L by dissolving 0.0250 grams of test substance in 100 mL of degassed water (ASTM Type II). Each sample was titrated against 0.005 N sodium hydroxide while maintained at a test temperature of 20±1°C. At least 10 incremental additions were made before the equivalence points and the titration was carried past the final equivalence point. Values of pK were calculated for a minimum of 10 points on the titration curve. Phosphoric acid and 4-nitrophenol were used as reference substances.
Result	:	Mean (N = 3) pKa values were 7.31 (SD = 0.0131) and 9.18 (SD= 0.0466) at 20°C
Remark	:	The results indicate that dissociation of the test substance will occur at environmentally-relevant pH values (approximately neutral) and at

physiologically-relevant pH values (approximately 1.2).
Supporting data for dissociation products:
Acid: Naphthenic acids exist as weak acids, with most pKa values being reported at about 5. At low pHs, they exist in their undissociated form and tend to partition onto solids. At high pHs, they exist in their dissociated form and become more mobile (Appendix IIA)

Reliability : [1] Reliable without restriction.
Reference : Lezotte, F.J. and W.B. Nixon, 2002. Determination of the dissociation constant of naphthenic acids, zinc salts, Wildlife International, Ltd. Study No. 534C-121, conducted for the Metal Carboxylates Coalition.

3.2.1 MONITORING DATA

Type of measurement :
Media :
Concentration : mg/l
Substance measured :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

3.3.1 TRANSPORT (FUGACITY)

Type :
Media :
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Year :
Test substance :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

3.5 BIODEGRADATION

Type :
Guideline/method :
Inoculum :
Concentration : related to
related to
Contact time :
Degradation : (±) % after day(s)
Result :
Kinetic of test subst. : % (specify time and % degradation)
%
%
%

		%
Control substance	:	
Kinetic	:	%
		%
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	**Supporting data for dissociation products:** **Acid:** Commercial mixtures of the sodium salts of naphthenic acids were shown to degrade and mineralize to CO_2 when inoculated with microbial populations indigenous to oil sands tailings. Approximately 50% of the organic carbon was converted to CO_2 over a 24-d period. Three of four model naphthenic acid compounds were also degraded by the enrichment cultures, with approximately 40-50% of the organic carbon converted to CO_2 over a 24-d period. Additional studies by Clemente et al. (2004) monitored the concentration and composition of naphthenic acids in aerobic biodegradation studies using sodium salts of naphthenic acids. Within 10 days of incubation with enrichment cultures on naphthenic acid-degraders, naphthenic acids concentration dropped from about 100 to <10 mg/L, accompanied by release of about 60% of the carbon as CO_2. GC/MS results indicated that the lower molecular weight acids (n = 5-13) were degraded more readily than high molecular weight acids. Clemente, J.S., M.D. Mackinnon, and P.M. Fedorak, 2004. Aerobic biodegradation of two commercial naphthenic acids preparations, Environ. Sci. Technol. 38:1009 – 1016.
Reliability	:	
Reference	:	

3.7 BIOCONCENTRATION

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	at °C
Concentration	:	
BCF	:	
Elimination	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

4.1 ACUTE TOXICITY TO FISH

Type	:	Static renewal
Guideline/method	:	FIFRA Guideline 72-1
Species	:	Bluegill sunfish (*Lepomis macrochirus*)
Exposure period	:	96 hr
NOEC	:	1.0 mg a.i./L
LC0	:	
LC50	:	1.5 mg a.i./L (1.1 – 2.0 mg a.i./L)
LC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	Yes
Year	:	1992
GLP	:	Yes
Test substance	:	Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid
Method	:	FIFRA Guideline 72-1, Acute toxicity test for freshwater fish
Method detail	:	The test material was prepared in acetone. Ten fish per test concentration (5 per replicate test vessel, 0.15 grams of biomass per liter) were exposed under static conditions to five concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in soft reconstituted water (hardness 38 mg/L as $CaCO_3$, pH 7.5) at a temperature of 19 - 21ºC. After 48 hours of exposure, all surviving fish were transferred to freshly prepared test solutions. This technique was used to maintain dissolved oxygen concentrations at acceptable levels.
Result	:	The mean measured concentrations averaged 94% of the nominal concentrations and were 5.0, 3.1, 1.7, 1.0 and 0.54 mg a.i./L. Complete mortality was observed at 96 hours at the two highest test concentrations. The 96-h LC50 was calculated to be 1.5 mg a.i/L (1.1 – 2.0 mg a.i./L). The NOEC was determined to be 1.0 mg a.i./L based upon sublethal effects (partial loss of equilibrium) seen in surviving fish exposed to 1.7 mg a.i./L.
Remark	:	**Supporting data for dissociation products:** **Acid:** Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox).
Reliability	:	[1] Reliable without restriction
Reference	:	Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) under Static Renewal Conditions. Springborn Laboratories, Inc. final report #92-3-4160, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio.

Type	:	Static
Guideline/method	:	FIFRA Guideline 72-1
Species	:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure period	:	96 hr
NOEC	:	0.39 mg a.i./L
LC0	:	
LC50	:	1.1 mg a.i./L (0.66 – 1.8 mg a.i./L)
LC100	:	
Other	:	

Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	Yes
Year	:	1992
GLP	:	Yes
Test substance	:	Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid
Method	:	FIFRA Guideline 72-1, Acute toxicity test for freshwater fish
Method detail	:	The test material was prepared in acetone. Ten fish per test concentration (5 per replicate test vessel, 0.21 grams of biomass per liter) were exposed under static conditions to five concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in soft reconstituted water (hardness 38 mg/L as $CaCO_3$, pH 7.4) at a temperature of 12 - 13ºC.
Result	:	The mean measured concentrations averaged 102% of the nominal concentrations and were 3.2, 1.8, 1.1, 0.66 and 0.39 mg a.i./L. Complete mortality was observed at 96 hours at the two highest test concentrations, with 50% mortality at the middle concentration and 0% mortality at the two lowest test concentrations. The 96-h LC50 was estimated by nonlinear interpolation to be 1.1 mg a.i/L (0.66 – 1.8 mg a.i./L). The NOEC was determined to be 0.39 mg a.i./L based upon sublethal effects (darkened pigmentation and partial loss of equilibrium) seen in several fish at the next highest test concentration.
Remark	:	**Supporting data for dissociation products:** **Acid:** Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox).
Reliability	:	[1] Reliable without restriction
Reference	:	Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Static Conditions. Springborn Laboratories, Inc. final report #92-3-4154, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Static
Guideline/method	:	FIFRA Guideline 72-2
Species	:	*Daphnia magna*
Exposure period	:	48 hr
NOEC	:	
EC0	:	
EC50	:	4.6 mg a.i./L (2.6 – 8.2 mg a.i/L)
EC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	Yes
Year	:	1992
GLP	:	Yes
Test substance	:	Zinc naphthenate, Lot # 24044-P, 98.9% active ingredient. Light brown viscous liquid
Method	:	FIFRA Guideline 72-2, Acute toxicity test for freshwater aquatic

		invertebrates
Method detail	:	The test material was prepared in acetone. Twenty daphnids (≤ 24 h old) per test concentration (5 per replicate test vessel) were exposed under static conditions to six concentrations of the test material, control, and solvent control (0.5 mL acetone/L) in fortified well water (hardness 170 mg/L as $CaCO_3$, pH 8.1) at a temperature of 20 – 21ºC.
Result	:	The mean measured concentrations averaged 71% of the nominal concentrations and were 35, 20, 14, 8.2, 4.6 and 2.6 mg a.i./L. Complete immobilization was observed at 48 hours at the four highest test concentrations, with 50% immobilization at the 4.6 mg/L concentration and 0% immobilization at the lowest test concentration. The 48-h EC50 was estimated by nonlinear interpolation to be 4.6 mg a.i/L (2.6 – 8.2 mg a.i./L). The NOEC was determined to be 2.6 mg a.i./L (no immobiliztaion or sublethal effects).
Remark	:	**Supporting data for dissociation products:** **Acid:** A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. (Bengtsson, B.E. and M. Tarkpea. 1983. The acute aquatic toxicity of some substances carried by ships. Mar. Pollut. Bull. 14:213-214). The zooplankton species Nephargoides maeoticus tolerated naphthenic acids concentrations up to only 0.15 mg/L (Dokholyan and Magomedov, 1984, cited in Clemente, J.S. and P.M. Fedorak, 2005, A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60:585-600).
Reliability	:	[1] Reliable without restriction.
Reference	:	Collins, M.K., 1992. Zinc Naphthenate – Acute Toxicity to Daphnids (*Daphnia magna*) under Static Conditions. Springborn Laboratories, Inc. final report #92-13-4089, submitted to The Naphthenate Council c/o Mooney Chemicals, Inc., Cleveland, Ohio.

4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Type	:	Acute Toxicity to Algae
Guideline/method	:	OECD #201
Species	:	*Pseudokirchneriella subcapitata*
Endpoint	:	Growth and mortality
Exposure period	:	72 h
NOEC	:	0.20 (growth); 0.79 (yield) as mg Zn naphthenate/L
LOEC	:	
EC0	:	
EC10	:	0.37 (growth); <0.15 (yield) as mg Zn naphthenate/L
EC50	:	0.78 (growth); 0.48 (yield) as mg Zn naphthenate/L
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	Nominal: As zinc naphthenate: 0.069, 0.17, 0.43, 1.1, 2.7 and 6.7 mg zinc naphthenate/L; As zinc: 0.010, 0.026, 0.064, 0.16, 0.40 and 1.0 mg Zn/L; Measured: As zinc naphthenate: 0.15, 0.20, 0.35, 0.79, 1.7 and 4.3 mg zinc naphthenate/L As zinc: 0.022, 0.030, 0.053, 0.12, 0.25 and 0.65 mg Zn/L)
Year	:	2007
GLP	:	Yes

Test substance	:	Zinc naphthenate, Lot No. B20P36, CAS No. 12001-85-3, reported to have a purity of 67% zinc naphthenate, 10.07% as zinc in the whole product, or 20.46% zinc in zinc naphthenate, tested as whole product (100%), was received from Alfa Aesar, Ward Hill, Massachusetts on 28 March 2006
Method	:	OECD # 201
Method detail	:	
Result	:	See above
Remark	:	**Supporting data for dissociation products:** **Acid:** The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 26.0 – 80.5 mg/L (Academy of Natural Sciences. 1960. Cited in the EPA ECOTOX Database 2005. http://www.epa.gov/ecotox).
Reliability	:	[1] without restriction
Reference	:	Zinc Naphthenate - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*. (2007). Conducted by Springborn Smithers Laboratories for the Metal Carboxylates Coalition. Study No. 13865.6121

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In vtro/in vivo	:	
Type	:	
Guideline/method	:	
Species	:	
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Route of administration	:	
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st.: 2nd.: 3rd.:
Toxic behavior	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.1.1 ACUTE ORAL TOXICITY

Type	:	Limit Test
Guideline/Method	:	
Species	:	Rat (albino)
Strain	:	Sherman-Wistar
Sex	:	Male and female
Number of animals	:	5 of each sex
Vehicle	:	None
Doses	:	Single dose of 5.0 g/kg given to all animals
LD50	:	> 5.0 g/kg
Year	:	1980
GLP	:	
Test substance	:	Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method	:	Described as similar to that in Federal Hazardous Substances Act regulations in 16 CFR 1500.3.
Method detail	:	One group of ten (5 male and 5 female) albino rats was used. Rats

		weighed between 200 and 300 grams each. Rats were deprived of food, but not water, overnight before dosing. Animals were dosed by direct administration into the stomach by means of a syringe and dosing needle. Following administration, the animals were allowed food and water *ad libitum* for the 14 day observation period during which rats were observed for signs of toxicity.
Result	:	There were no mortalities. Shortly after dosing, the animals were slightly letharegic and ruffled. They appeared normal after 24 hours. Gross pathological examination revealed nothing remarkable.
Remark	:	**Supporting data for dissociation products:** **Acid:** Other data for rats includes an LD50 of 3.0 g/kg bw for naphthenic acid fraction from crude kerosene acids and 5.2 g/kg bw for naphthenic acid fraction from mixed crude oils (Rockhold, 1955, as cited in Appendix A of Appendix II). An oral acute toxicity test with a mixture of naphthenic acids isolated from Athabasca oil sands produced appetite suppression, hepatoxicity and cardiovascular effects with a single dose of 300 mg/kg. (Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355). **Metal:** Acute oral toxicity in rodents exposed to zinc is low, and the level at which zinc produces no adverse effect in rats is approximately 160 mg/kg body weight (WHO, 2001, Environmental Health Criteria 221, Zinc). Of the compounds zinc nitrate, zinc sulfate, zinc chloride and zinc acetate, zinc acetate was the most toxic, with oral LD50 values of 237 mg Zn/kg bw (rat) and 86 mg Zn/kg bw (mouse). The LD50 for zinc chloride in an oral exposure was reported to be 528 mg Zn/kg bw in rats and 605 mg Zn/kg bw in mice (ATSDR, 1994, Toxicological Profile for Zinc).
Reliability	:	[2] Reliable with restrictions. Basic data given: comparable to guidelines.
Reference	:	Biosearch, Inc. (1980). Fungitrol Zinc 8% Toxicological Studies. Project number 80-2171A. Submitted to Tenneco Chemicals. [Available from the National Technical Information Service in microfiche OTS05151131, „Eight toxicological studies of naphthenic acids, zinc salts with attachments and cover letter dated 072187“][Subsequently referenced as Biosearch (1980)]

Type	:	Limit test
Guideline/Method	:	Oral Toxicity Single Dose, EPA 40 CFR 163.81-1 (Proposed)
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Five males and five females, weighing 200 – 300 grams each
Number of animals	:	10
Vehicle	:	
Doses	:	Single dose of 5 g/kg administered to all animals
LD50	:	> 5 g/kg
Year	:	1985
GLP	:	No
Test substance	:	2% zinc naphthenate, in mineral spirits solvent. Sample density 0.82 g/mL
Method	:	Oral Toxicity Single Dose, EPA 40 CFR 163.81-1 (Proposed)
Method detail	:	Food (but not water) withheld 24 hours prior to dosing. Following dosing by gavage, food and water allowed *ad libitum*. Animals observed twice daily for 14 days, weight recorded after 7 and 14 days. All animals autopsied.
Result	:	Lethargy, piloerection and nasal discharge were observed in some animals following intubation. 1/5 females and 0/5 males died (death at 30 hours following intubation). All surviving animals appeared normal at 48 hours and no abnormal behavioral or physical symptoms were observed during the remainder of the observation period. Hemhorragic lungs, dark kidneys and pale spleen in the dead animal; all other animals had normal tissues and organs at autopsy.

Remark	:	
Reliability	:	[2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance.
Reference	:	Hoster, S., 1985. Acute Toxicology – Oral, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.
Type	:	Oral LD50
Guideline/Method	:	
Species	:	Rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	Stoddard-type solvent
Doses	:	
LD50	:	> 6.0 g/kg
Year	:	
GLP	:	
Test substance	:	Zinc naphthenate containing 8.0% zinc
Method	:	Smyth & Carpenter (1944)
Method detail	:	Dosing by gavage
Result	:	
Remark	:	
Reliability	:	[4] Not reliable. Documentation insufficient for assessment.
Reference	:	Rockhold, W.T. 1955. Toxicity of naphthenic acids and their metal salts. A.M.A. Arch. Indust. Health. 12: 477-482.

5.1.2 ACUTE INHALATION TOXICITY

Type	:	Limit test
Guideline/method	:	
Species	:	Rat (albino)
Strain	:	
Sex	:	Male and female
Number of animals	:	5 of each sex
Vehicle	:	Mineral spirits
Concentrations	:	A single concentration of 11.6 mg/L was administered to all animals
Exposure time	:	4 hr
LC50	:	>11.6 mg/L (for a 50% w/v suspension in mineral spirits)
Year	:	1980
GLP	:	Yes (per EPA's proposed GLP regulations at the time)
Test substance	:	Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method	:	Similar to that proposed in 40 CFR 163.81-3 (August 22, 1978).
Method detail	:	Animals were exposed to an aerosol of the test material inside a 260 liter plexiglass exposure chamber for four hours (flow rate of 20 L per minute). Following the exposure period, animals were returned to their cages and observed for a 14-d period. Signs of toxicity and mortalities were noted. The aerosol was generated by a six jet Collision nebulizer. Particle size of the aerosol was determined using an Andersen Sampler cascade impactor. The mass median diameter of particles was 0.54 μm, within the respirable range. The concentration of particles was 0.42 mg/L.
Result	:	There were no mortalites of exposed animals. Animals appeared depressed and ruffled within 18 to 24 hours after exposure, but returned to normal after 48 hours. Gross pathological examination revealed nothing remarkable.
Remark	:	

Reliability	:	[2] Reliable with restrictions. Basic data given: comparable to guidelines.
Reference	:	Biosearch, Inc. (1980).
Type	:	Limit test
Guideline/method	:	Inhalation toxicity – EPA (40 CFR 163.81-3)
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male and female, weighing 200 – 300 grams each
Number of animals	:	5 of each sex for the exposure, 5 of each sex for the control
Vehicle	:	Mineral spirits
Concentrations	:	A single concentration (25.2 mg/L nominal, 0.72 mg/L assayed) was administered to all animals.
Exposure time	:	4 hr
LC50	:	
Year	:	1985
GLP	:	No
Test substance	:	2% zinc naphthenate in mineral spirits solvent
Method	:	Inhalation toxicity - EPA (40 CFR 163.81-3)
Method detail	:	Animals were exposed to an aerosol of the test material inside a 392 liter plexiglass exposure chamber for four hours (flow rate 20 L/min.). Sample (1000 g) was sprayed into the chamber with a Burgess Thermo Model F-982. Sample was sprayed for 15 seconds at 5 minute intervals for the first 15 minutes and then 5 seconds at 5 minute intervals for the remaining time. Following the exposure period, animals were returned to their cages and observed twice daily for a 14-d period. Signs of toxicity and mortalities were noted, and weights taken at 2,3,4 and 7 days. A group of 10 rats was held for a two week observation period under the same conditions. Particle size of the aerosol was determined using an Andersen Sampler, with 87-88% of the particles 9-10 μm or larger.
Result	:	There were no mortalites of exposed animals. Animals appeared normal at the end of the exposure period and for the duration of the observation period. Autopsies indicated one exposed animal and one control animal with hypervacuolization of the center of the right kidney; all other tissues and organs appeared normal.
Remark	:	
Reliability	:	[2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance.
Reference	:	Hoster, S., 1985. Acute Toxicology – Inhalation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.

5.1.3 ACUTE DERMAL TOXICITY

Type	:	Limit test
Guideline/method	:	
Species	:	Rabbit (albino)
Strain	:	
Sex	:	Male and female
Number of animals	:	5 of each sex
Vehicle	:	None
Doses	:	A single dose of 2.0 g/kg was administered to all animals.
LD50	:	>2.0 g/kg
Year	:	1980
GLP	:	Yes (per EPA's proposed GLP regulations at the time)
Test substance	:	Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method	:	Similar to that proposed in 40 CFR 163.81-2 (August 22, 1978).

Method detail	:	Animals weighed between 2.0 and 3.0 kg. All animals had their backs clipped free of hair 24 hours prior to testing. All animals had their backs abraded prior to dosing. Test material was applied to the back of each animal and covered with a large gauze patch. An impervious material was then wrapped snugly around the trunk of each animal. The dressings were removed after 24 hours and any excess test material was removed. Animals were observed for a period of 14 days for signs of toxicity.
Result	:	There were no mortalities in the test. Very substantial skin irritation was noted throughout the observation period, but no other untoward symptoms were observed. Gross pathological examination of all survivors revealed nothing remarkable.
Remark	:	
Reliability	:	[2] Reliable with restrictions. Basic data given: comparable to guidelines.
Reference	:	Biosearch, Inc. (1980).
Type	:	Limit test
Guideline/method	:	Dermal Toxicity – EPA (40 CFR 163.81-2)
Species	:	Rabbit (albino)
Strain	:	New Zealand
Sex	:	Male and female
Number of animals	:	5 of each sex (exposed); 5 of each sex (untreated control)
Vehicle	:	None
Doses	:	A single dose of 2.0 g/kg was administered to all animals.
LD50	:	>2.0 g/kg
Year	:	1985
GLP	:	No
Test substance	:	2% zinc naphthenate, in mineral spirits solvent. Sample density 0.83 g/mL
Method	:	Dermal Toxicity – EPA (40 CFR 163.81-2)
Method detail	:	The trunks of the animals were clipped free of hair and abraded prior to dosing. An impervious sleeve was wrapped around the trunk and the dose introduced under the sleeve. At the end of 24 hours, the sleeve was removed, skin reactions noted, and any excess test material removed. Animals were observed for a period of 14 days for signs of toxicity. Weight changes were recorded at 7 days. Gross pathology performed at study termination.
Result	:	There were no mortalities in the test. All exposed animals exhibited slight erythema at 24 hours and 48 hours. By 72 hours, only 3 animals showed slight erythema and by day 7 all signs of irritation had subsided. No edema was observed. One animal showed weight loss and one showed diarrhea. No other untoward symptoms were observed. Gross pathological examination indicated congested spleen in 2 exposed animals, pale thin spleen in one exposed animal, streak in the liver in one exposed animal, and an abscess under the skin in one animal. All other organs and tissues appeared normal; four autopsied control animals demonstrated normal pathology.
Remark	:	**Supporting data for dissociation products:** **Acid:** No deaths occurred in an acute dermal toxicity study. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively.
Reliability	:	[2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance.
Reference	:	Hoster, S., 1985. Acute Toxicology – Dermal, 2% Zinc Naphthenate in

Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.

5.2.1 SKIN IRRITATION

Type	:	Primary skin irritation
Guideline/method	:	
Species	:	Rabbit (albino)
Strain	:	
Sex	:	
Concentration	:	
Exposure	:	0.5 ml of undiluted test material
Exposure time	:	24 hr
Number of animals	:	Six
Vehicle	:	None
Classification	:	Study 1: primary skin irritant; Study 2: skin irritant
Year	:	1980
GLP	:	Yes (per EPA's proposed GLP regulations at the time)
Test substance	:	Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method	:	Similar to that proposed in 40 CFR 163.81-5 (August 22, 1978).
Method detail	:	The test was conducted twice. After clipping, a 0.5 ml sample of the test material was applied to areas of intact and abraded skin on six albino rabbits for a period of 24 hours. Test material was held in place by gauze patches secured with an impervious material wrapped around the torso of each animal. Examination and scoring (Draize method) for erythema, eschar, and edema was conducted at 24 and 72 hours.
Result	:	Results were similar for both intact and abraded skin and at both time points. Scores were similar for the primary endpoints. The primary irritation scores were 6.29 and 4.29 for the first and second tests, respectively.
Remark	:	**Supporting data for dissociation products:** **Acid:** Moderately to severely irritating to rabbits. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively
Reliability	:	[2] Reliable with restrictions. Basic data given: comparable to guidelines.
Reference	:	Biosearch, Inc. (1980).

Type	:	Primary skin irritation
Guideline/method	:	Skin Irritation Test – EPA (40 CFR 163.81-5)
Species	:	Rabbit (albino)
Strain	:	New Zealand
Sex	:	Not specified
Concentration	:	
Exposure	:	0.5 ml of undiluted test material
Exposure time	:	24 hr
Number of animals	:	Six
Vehicle	:	
Classification	:	Slight irritation at 72 hours but subsided by 96 hours
Year	:	1985
GLP	:	No
Test substance	:	2% zinc naphthenate, solvent.
Method	:	Skin Irritation Test – EPA (40 CFR 163.81-5)
Method detail	:	The trunk of each animal was clipped free of hair. After clipping, a 0.5 ml

		sample of the test material was applied to two areas of intact and two areas of abraded skin on six albino rabbits for a period of 24 hours. Test material was held in place by gauze patches secured with an impervious material wrapped around the torso of each animal. Examination and scoring for erythema, eschar, and edema was conducted at 24, 72 and 96 hours.
Result	:	At 24 hours, no erythema was observed but two animals had slight to moderate edema on abraded skin. At 72 hours, 5 animals exhibited slight erythema but no animals exhibited edema. By 96 hours, all signs of irritation had subsided.
Remark	:	
Reliability	:	[2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance.
Reference	:	Hoster, S., 1985. Acute Toxicology – Skin Irritation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.

5.2.2 EYE IRRITATION

Type	:	Primary eye irritation
Guideline/method	:	
Species	:	Rabbit (albino)
Strain	:	New Zealand White
Sex	:	Not specified
Concentration	:	
Dose	:	0.1 ml of undiluted test material
Exposure time	:	
Number of animals	:	Six
Vehicle	:	None
Classification	:	Not a primary ocular irritant
Year	:	1980
GLP	:	Yes (per EPA's proposed GLP regulations at the time)
Test substance	:	Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method	:	Similar to that proposed in 40 CFR 163.81-4 (August 22, 1978).
Method detail	:	A 0.1 ml sample of the material was instilled into the right eyes of six adult rabbits. Left eyes were untreated and served as controls. The test material was not washed from the eyes. The treated eyes were examined and scored according to Draize scale at one, two, three, five, and seven days following instillation of the test material.
Result	:	Total ocular irritation scores ranged from 4 to 8 (avg. = 7.0) for individual animals at 24 hours after instillation. Total ocular irritation scores were zero for all animals at all subsequent time points.
Remark	:	**Supporting data for dissociation products:** **Acid:** Raw naphthenic acid derived from kerosene was judged to be an irritant. In a later summary report, eye irritation was judged to be moderate
Reliability	:	[2] Reliable with restrictions. Basic data given: comparable to guidelines.
Reference	:	Biosearch, Inc. (1980).

Type	:	Primary eye irritation
Guideline/method	:	Skin Irritation Test – EPA (40 CFR 163.81-4 proposed)
Species	:	Rabbit (albino)
Strain	:	
Sex	:	Not specified
Concentration	:	
Dose	:	0.1 ml of undiluted test material
Exposure time	:	
Number of animals	:	Nine (6 exposed and 3 control)

Vehicle	:	None
Classification	:	Not an irritant
Year	:	1985
GLP	:	No
Test substance	:	2% zinc naphthenate, solvent.
Method	:	Skin Irritation Test – EPA (40 CFR 163.81-4 proposed)
Method detail	:	A 0.1 ml sample of the material was instilled into the right eyes of six adult rabbits. In these six animals, the test material was not washed from the eyes. Left eyes were untreated and served as controls. In three other adult rabbits, the test material was instilled in the same manner but each eye was subsequently flushed with lukewarm water no sooner than 20-30 seconds after instillation. The treated eyes were examined and scored for damage to the cornea, iris and conjunctiva at 1, 2, 3, 4 and 7 days after treatment.
Result	:	All ocular irritation scores were zero at all time points. No irritation was observed.
Remark	:	
Reliability	:	[2] Reliable with restriction. Basic data given, comparable to guidelines. Limited description of test substance.
Reference	:	Hoster, S., 1985. Acute Toxicology – Eye Irritation, 2% Zinc Naphthenate in Mineral Spirits Solvent, Applied Biological Sciences Laboratory, prepared for Mooney Chemicals Inc.

5.4 REPEATED DOSE TOXICITY

Type	:	90-day dermal toxicity
Guideline/method	:	FIFRA 82-3 and OECD 411
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male and female
Number of animals	:	10 of each sex per treatment group
Route of admin.	:	Dermal
Exposure period	:	6 hours per day for 13 weeks
Frequency of treatment	:	Once per day; 5 days per week for 13 weeks
Post exposure period	:	None
Doses	:	100, 300, and 1000 mg/kg/day
Control group	:	Yes
NOAEL	:	300 mg/kg/day excluding dermal irritation as an endpoint
LOAEL	:	1,000 mg/kg/day excluding dermal irritation as an endpoint
Other	:	Dermal irritation was present at the application site in all groups, including control. Irritation increased in a dose-related manner.
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (Purity = 98.9%; 14.3% zinc)
Method	:	
Method detail	:	Test substance was dissolved in light mineral oil at a concentration of 50% by weight and administered onto the clipped intact dorsal skin (right flank) of each animal. After application, each test site was wrapped with a gauze binder and the dressing secured with Deriform® tape. At the end of a 6-hour exposure period, the dressings were removed and the test sites were wiped with disposable paper towels moistened with mineral oil. The concurrent control group received the vehicle (mineral oil) on a comparable regimen at a dose volume equal to the amount of vehicle received by the highest dose group.
Result	:	No treatment-related clinical signs or effects on mortality were apparent in the study; however, dermal irritation (including moderate and severe grades of erythema and edema, as well as fissuring) was observed in a dose-

related manner. Severe signs of skin irritation such as eschar and blanching were not observed. A tolerance developed to the irritating effects of the test substance and the incidences of severe edema, erythema and fissuring were lower during the final weeks of the study. Histopathologic evaluation of the application sites revealed treatment-related lesions characterized by hyperkeratosis of the epidermal surface and dermal hyperplasia. Body weight means of both male and female rabbits in the 1000 mg/kg/day group were lower than control means throughout the study. Relative mean kidney and adrenal weights of the high dose group's animals were significantly above the control mean. No treatment-related effects were apparent in the serum chemistry values. A slight increase in neuturophils in the high dose group was the only alteration in clinical pathological parameters indicative of a treatment-related effect.

Remark :
Reliability : (1) Reliable without restrictions.
Reference : Tomkins, E.C. 1990. 90-Day dermal study in rabbits with zinc naphthenate. WIL Research Laboratories. Lab Study No. WIL-153006.

Type : Contact dermal irritation / Sensitization
Guideline/method :
Species : Guinea pig (albino)
Strain :
Sex : Male
Number of animals : 10
Route of admin. : Dermal
Exposure period : See method details below
Frequency of treatment : See method details below
Post exposure period : See method details below
Dose : 0.5 ml of 10% w/v suspension in mineral spirits
Control group : None
NOAEL :
LOAEL :
Other :
Year : 1980
GLP : Yes (per EPA's proposed GLP regulations at the time)
Test substance : Fungitrol Zinc 8% fungicide (Lot #LPP 3000-4)
Method :
Method detail : A 0.5 ml sample of test material was applied to intact skin test sites on 10 guinea pigs. A gauze patch was used to hold the test substance in place. After a 24-hour contact period, the patch was removed and the animals were allowed to rest for one day. Following the rest period, another application was applied to the same skin site using a fresh sample. This sequence was repeated for a total of ten induction applications. After the tenth application, the animals were rested for a two-week period. Following this period, a challenge application was placed at skin sites differing from the original test sites. The challenge application was removed after 24 hours. Sites were examined for irritation using the Draize scale 24 hours after each induction application, and 24 and 48 hours after the challenge application.
Result : The test material produced well defined erythema and very slight edema during the induction period. Similar or slightly less severe effects were noted after the challenge dose. Based on study results, the test material appeared to be a primary skin irritant and fatiguing agent, and possibly a sensitizing agent in the guinea pig.
Remark :
Reliability : [2] Reliable with restrictions. Basic data given: comparable to guidelines.

Reference	:	Biosearch, Inc. (1980).

5.5 GENETIC TOXICITY 'MUTAGENICITY'

Type	:	L5178Y (TK+/TK-) Mouse lymphoma mutagenesis
Guideline/method	:	FIFRA 84-2
System of testing	:	Suspension / plate
Species	:	Mouse
Strain	:	L5178Y (TK+/TK-)
Test concentrations	:	Initial assay: 1.3 to 100 μg/ml; Confirmatory assay: 7.5 to 75 μg/ml
Cytotoxic concentr.	:	100 μg/ml for nonactivated cultures; 1000 μg/ml for activated cultures
Metabolic activation	:	Rat liver S-9 fraction, induced with Aroclor 1254
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn)
Method	:	Clive and Spector, 1975 (Mutation Res. 31:17-29)
Method detail	:	Ethanol was used as the solvent for preparing dilutions of the test substance.
Result	:	Positive findings (mutant frequencies at least twice the frequency of the controls), both with and without metabolic activation, were found in the initial and confirmatory assays. A dose-dependent response was seen in the treated cultures both with and without metabolic activation. Colony sizing data indicated an increase in the proportion of small mutant colonies from cultures treated with the test substance, suggesting that it may show clastogenic activity. All criteria for a valid test were met.
Remark	:	**Supporting data for similar salts:** Similar mouse lymphoma tests with the calcium and copper salts of naphthenic acids were also positive both with and without metabolic activation. However, copper naphthenate produced negative results in the Ames Assay with *Salmonella typhimurium* both with and without metabolic activation. (Reference: Short-term test program sponsored by the Division of Cancer Etiology, National Cancer Institute, Dr. David Longfellow, Project Officer. Cited in Chemical Carcinogenesis Research Information System, National Library of Medicine: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS)
Reliability	:	(1) Reliable without restrictions.
Reference	:	Harbell, H.W. 1990. L5178Y TK+/- mouse lymphoma mutagenesis assay with confirmation – test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.701.

Type	:	Unscheduled DNA Synthesis
Guideline/method	:	FIFRA 84-4
System of testing	:	Primary hepatocytes
Species	:	Rat
Strain	:	Harlan Sprague-Dawley
Test concentrations	:	0.015 to 35 μg/ml (8 dose levels)
Cytotoxic concentr.	:	15 μg/ml
Metabolic activation	:	No
Year	:	1989
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn)
Method	:	Williams, 1979 (In Chemical Mutagens, Vol. VI, DeSerres, F.J. and A. Hollander, eds., Plenum Press, pp 61-79)
Method detail	:	Ethanol was used to dissolve the test substance and as a solvent control. DMBA was used as a positive control. A parallel cytotoxicity test was conducted to determine the relative toxicity of the test substance.

Result	:	The test substance did not cause a significant increase in unscheduled DNA synthesis as measured by the mean number of net nuclear grain counts at any dose level. All criteria for a valid test were met.
Remark	:	
Reliability	:	(1) Reliable without restriction.
Reference	:	Curren, R.D. 1989. Unscheduled DNA synthesis in rat primary hepatocytes - test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.380.

5.6 GENETIC TOXICITY 'CHROMESOMAL ABERRATION

Type	:	Chromosome aberration assay
Guideline/method	:	FIFRA 84-2
System of testing	:	Chinese hamster ovary cells
Species	:	Hamster
Strain	:	Chinese
Test concentrations	:	Initial assay: 5 to 80 µg/ml for nonactivated cultures;10 to 160 µg/ml for activated cultures; Confirmatory assay: 80 to 200 µg/ml for nonactivated cultures; 60 to 140 µg/ml for activated cultures
Cytotoxic concentr.	:	80 µg/ml
Metabolic activation	:	Yes, with Aroclor induced S-9 fraction from male Sprague-Dawley rats
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (Purity = 98.9%; 14.3% Zn)
Method	:	
Method detail	:	Ethanol was used to dissolve the test substance and as a solvent control. Triethylenemelamine and cyclophosphamide were used as positive controls. Whenever possible, a minimum of 100 metaphase spreads (50 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations.
Result	:	Zinc naphthenate produced positive results in the CHO cytogenetics assay. Toxicity was a limiting factor in the analysis of test concentrations in both the nonactivated and S-9 activated studies. The percentage of cells with structural chromosome aberrations was significantly increased, in a dose-responsive manner, at all test concentrations analyzed for both the S-9 activated and the nonactivated test systems.
Remark	:	
Reliability	:	(1) Reliable without restriction.
Reference	:	Putman, D.L. and M.J. Morris. 1990. Chromosome aberrations in Chinese hamster ovary (CHO) cells - test article zinc naphthenate. Microbiological Associates, Inc. Lab Study No. T9036.337.

5.8.2 DEVELOPMENTAL TOXICITY

Type	:	Teratology / developmental toxicity
Guideline/method	:	
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Female
Route of admin.	:	Oral
Exposure period	:	Day 6 through 15 of gestation
Frequency of treatment	:	Daily
Duration of test	:	Mating until day 20 of gestation
Doses	:	94, 188, and 938 mg/kg/day
Control group	:	Yes (received 3.75 mL/kg/day of corn oil)

NOAEL maternal tox.	:	188 mg/kg/day
NOAEL teratogen.	:	188 mg/kg/day
Other	:	LOAEL was 938 mg/kg/day for maternal toxicity
Other	:	LOAEL was 938 mg/kg/day for toxicity to fetuses
Other	:	
Year	:	1991
GLP	:	Yes
Test substance	:	Zinc naphthenate, technical, containing 13.7% zinc. Dosed in corn oil.
Method	:	Standing Operating Procedure No. 25, Teratology Study in Rats, July 1981, Toxicology Division, U.S. Army Environmental Hygiene Agency.
Method detail	:	Doses were set based on results of a pilot study. There were at least 33 positively mated females in each dose group. Females were sacrificed on day 20 of gestation. Each uterus was exposed and counts were made of corpora lutea, implantation sites, resorptions, and fetuses. Fetuses were preserved and examined for either skeletal (even-numbered fetuses) or soft tissue (odd numbered fetuses) malformations.
Result	:	Oral administration of zinc naphthenate to rats during the major period of organogenesis did not result in teratogenic effects. Transient maternal toxicity was confined to the highest dosage group (938 mg/kg/day) and consisted of lethargy and lower body weight gain. Maternal treatment at that dosage level also produced a higher incident of resorptions and lower average fetal body weights. Dams receiving zinc naphthenate at either 94 or 188 mg/kg/day were not adversely affected, nor were their developing fetuses. Compared to controls, there was an increase in the incidence of variants (minor morphological deviations) in all treatment groups; however, there was not a dose-response for this effect. It was concluded that zinc naphthenate is not teratogenic and does not cause developmental toxicity at doses that are not maternally toxic.
Remark	:	
Reliability	:	[1] Reliable without restriction. Comparable to guideline study.
Reference	:	Angerhofer, R.A., M.W. Michie, M.P. Barlow, and P.A. Beall. 1991 Phase 4, Toxicological Study No. 75-51-0497-91, Assessment of the developmental toxicity of zinc naphthenate in rats, June 1985 – July 1988. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD. NTIS No. ADA235308.

Type	:	Oral administration
Guideline/method	:	FIFRA 83-3
Species	:	Rat
Strain	:	Sprague-Dawley Crl:CDBR
Sex	:	Female
Route of admin.	:	Oral
Exposure period	:	Day 6 through 15 of gestation
Frequency of treatment	:	Daily
Duration of test	:	Mating until day 20 of gestation
Doses	:	50, 250, and 500 mg/kg/day
Control group	:	Yes (received 10 mL/kg/day of corn oil)
NOAEL maternal tox.	:	250 mg/kg/day (excluding marginal clinical signs)
NOAEL teratogen.	:	500 mg/kg/day
Other	:	LOAEL was 500 mg/kg/day for maternal toxicity (based on clinical signs and slightly reduced food consumption)
Other	:	LOAEL for fetuses was above the highest dose tested
Other	:	
Year	:	1990
GLP	:	Yes
Test substance	:	Technical grade zinc naphthenate (purity = 98.9%; 14.3% Zn).

Method	:	Oral gavage
Method detail	:	Doses were set based on results of a range-finding study. The test substance was dissolved in corn oil and administered by gastric gavage at a dose volume of 10 ml/kg. There were 25 positively mated females in each dose group. Females were sacrificed on day 20 of gestation for a scheduled Cesarean section. The uteri and ovaries were examined and the location and numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Fetuses were weighed, sexed, and examined for external, skeletal and soft tissue malformations and developmental variations.
Result	:	Maternal survival was not adversely affects in the study and no indication of maternal toxicity was apparent at a dose level of 50 mg/kg/day. Clinical signs of toxicity observed in the high dose females included anogenital and/or urogenital staining, staining around the mouth, and salivation. Some of the same clinical signs were also seen in females at the mid-dose level, although the incidence was greatly reduced. No adverse effects were apparent on body weight data or gravid uterine weight data although food consumption was slightly reduced in the high dose group. Intrauterine growth and survival were not adversely affected at any of the treatment levels. The nature and frequency of fetal malformations and developmental variations expressed appeared to be spontaneous in origin.
Remark	:	**Supporting data for dissociation products:** **Acid:** Results are highly consistent with the developmental toxicity study conducted on zinc naphthenate by the U.S. Army Environmental Hygiene Agency (see above).
Reliability	:	[1] Reliable without restriction. Comparable to guideline study.
Reference	:	Nemec, M.D. 1990. A developmental toxicity study of zinc naphthenate in rats. WIL Research Laboratories, Inc. Lab Study No. WIL-153004.

5.8.3 TOXICITY TO REPRODUCTION

Type	:	Two generation, oral administration
Guideline/method	:	
In vitro/in vivo	:	In vivo
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male and female
Route of admin.	:	Diet
Exposure period	:	Two generations
Frequency of treatment	:	Continuous in diet
Duration of test	:	Through weaning of second (F2) generation of offspring
Doses	:	500, 1000, or 5000 ppm in diet
Control group	:	Yes
Year	:	1991
GLP	:	Yes
Test substance	:	Zinc naphthenate, technical, containing 13.7% zinc. Dosed in corn oil.
Method	:	Standing Operating Procedure, Reproduction Study in Rats, August 1986 revision, Toxicology Division, U.S. Army Environmental Hygiene Agency.
Method detail	:	Rats were fed zinc naphthenate for 10 weeks prior to mating of the parental (P) generation. Feeding of the treated diet was continued during mating, gestation, and lactation for both the P and F1 generations. Body weights and feed consumption were measured three times per week during the exposure period. Animals were checked daily for toxic signs. After sacrifice, animals were examined grossly and target organ tissues removed for histopathologic examination. Individual body weights, abnormalities,

		mortalities, and total litter weights for F1 pups were noted on days 0, 4, 7, 14, and 21 post partum.
Results	:	The continuous diets of zinc naphthenate employed in the study produced no adverse effects on reproductive function of rats over two generations. Rats fed a diet of 5,000 ppm experienced a significant weight loss (or reduced weight gain), but this effect had no subsequent effect on mating or viability of offspring over two generations. It is concluded that zinc naphthenate does not produce adverse effects on reproduction at dietary levels that are not maternally or paternally toxic. The NOAEL for all endpoints in this study was 1,000 ppm in the diet.
Remark	:	
Reliability	:	[1} Reliable without restriction. Comparable to guideline study.
Reference	:	Michie, M.W., Angerhofer, R.A., M.P. Barlow, and P.A. Beall. 1991 Phase 5, Effects of ingestion of zinc naphthenate on reproductive function of rats, Toxicological Study No. 75-51-0497-91. U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, MD. NTIS No. ADA235224.

6.0 OTHER INFORMATION

6.1 Carcinogenicity

No adequate experimental evidence has been found to indicate that zinc salts administered orally or parenterally are tumorigenic. (WHO, 2001, Environmental Health Criteria 221, Zinc).

6.2 Skin sensitization

Zinc sulfate is not a skin sensitizer in animals. (Risk Assessment for Zinc Metal, 2001, draft).

1. General Information

ID 1338-24-5

Date December 15, 2005

1.0 SUBSTANCE INFORMATION

Generic Name	:	
Chemical Name	:	Naphthenic acids
CAS Registry No.	:	1338-24-5
Component CAS Nos.	:	
EINECS No.	:	215-662-8
Structural Formula	:	
Additional description	:	Naphthenic acid is mixture of various carboxylic acids which occur naturally in crude petroleum. The most common class of acid is derived from cyclopentane and has the general formula CnH2n-202, where n = 8 to 12. This basic cyclopentane structure can be more or less highly alkylated. Other classes of acids include simple paraffinic acids of the general formula CnH2n02 where n = 5 to 8, and acids with larger more complicated molecules of the general formula CnH2-402, where n = 13 to 23. The classes and proportions of individual naphthenic acids in the overall mix vary according to the origin of the crude oil.
Molecular Weight	:	Generally between 140 and 450
Synonyms and Tradenames	:	
References	:	AGS Chemicals Ltd., 2003, Product Information, Naphthenic Acid; Headley, J.V. and D.W. McMartin, 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(8):1989 -2010.

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	-35 to +2°C
Decomposition	:	at °C
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	Commercially available naphthenic acid
Method	:	
Method detail	:	
Result	:	
Remark	:	A range of melting points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated melting points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Melting points calculated using EPIWIN v3.10 ranged from 117ºC to 160ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a melting point range of -35º to +2ºC (AGS Chemicals Ltd., 2005).
Reliability	:	
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C); AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid ().

2.2 BOILING POINT

Type	:	
Guideline/method	:	
Value	:	140ºC to 200ºC
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	Commercially available naphthenic acid
Method	:	
Method detail	:	
Result	:	
Remark	:	A range of boiling points would be expected, based upon the hydrocarbon composition of the specific naphthenic acid mixture. Estimated boiling points were calculated for one to four ring cycloalkyl naphthenic acid structures with molecular weights ranging from 260 to 320; these dominate profiles of natural naphthenic acids in extracts of Athabasca oil sands. Boiling points calculated using EPIWIN v3.10 ranged from 233ºC to 375ºC for these structures (Appendix C). In contrast, structural profiles of commercial naphthenic acids have been shown to differ substantially from natural extracts (Rogers et al., 2002, cited in Appendix C). Product literature for commercially available naphthenic acid provides a boiling point range of 140º to 200ºC (AGS Chemicals Ltd., 2005).
Reliability	:	
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C); AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid.

2.3 DENSITY

Type	:	
Guideline/method	:	
Value	:	0.91 to 0.96 g/cm^3 at 15ºC
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference		AGS Chemicals Ltd., 2005, Product Information, Naphthenic Acid (http://www.ags-chemicals.com)

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	It was estimated using EPIWIN v.310 that the vapor pressures of the components of naphthenic acid mixtures would be near or below the measurable limits cited in standard guideline methods and thus, the total vapor pressure of naphthenic acids is expected to be exceedingly low (Appendix C)
Reliability	:	
Reference	:	API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C)

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	
pH value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Using EPIWIN v3.10, partition coefficients were estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting log Kow values ranged from 5.1 to 9.2. Mixtures of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show lower log Kow values than those

presented. (Appendix C)

Reliability :

Reference : API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C)

2.6.1 SOLUBILITY IN WATER

Type :
Guideline/method :
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
PKa : at °C
Description :
Stable :
Deg. product :
Year :
GLP :
Test substance :
Deg. products CAS# :
Method :
Method detail :
Result :
Remark : Using EPIWIN v3.10, water solubility was estimated for a range of molecular weight naphthenic acids spanning the molecular weights and numbers of cycloalkane rings reported to predominate in Athabasca oil sands extracts. Resulting water solubility estimates ranged from 0.0003 to 2.1 mg/L. Mixtures of naphthenic acids with a significant proportion of structures with molecular weights below 250 will likely show greater water solubilities than those presented. (Appendix C)
Reliability :
Reference : API, 2003, Robust Summary of Information on Reclaimed Substances: Naphthenic Acid (attached as Appendix C);

2.7 FLASH POINT

Type :
Guideline/method :
Value :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference : .

3.1.1 PHOTODEGRADATION

Type		
Guideline/method	:	
Light source	:	
Light spectrum	:	
Relative intensity	:	based on
Spectrum of substance	:	lambda (max, >295nm) :
		epsilon (max) :
		epsilon (295) :
Conc. of substance	:	at °C
DIRECT PHOTOLYSIS		
Halflife (t1/2)	:	
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	
Degradation	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	Three naphthenic acid mixtures (two commercially-available and one extracted from an Athabasca Oil Sands tailings pond) as well as three individual naphthenic acids: 4-methylcyclohexaneacetic acid (4-MCHAA), 4-methylcyclohexanecarboxylic acid (4-MCHCA), and 3-methylcyclohexane-carboxylic acid (3-MCHCA).
Deg. products CAS#	:	
Method	:	
Method detail	:	Experiments were conducted with natural sunlight, artificial solar radiation in growth chambers using an incandescent and fluorescent lamp canopy, artificial UV-range solar radiation in quartz annular photochemical cells, and UV-254 ultraviolet lamps in quartz annular photochemical cells. All aqueous solutions of naphthenic acids were prepared in Athabasca Rivver water and 1 mL aliquots collected at selected time intervals to assess photochemical degradation as well as toxicity changes. Concentrations were 0.5 to 125 mg/L depending upon the compound or mixture under study. Control reactors were monitored simultaneously in the absence of UV light in natural water and in both the absence and presence of UV light in reagent water. The production of hydroxyl radicals during photolysis was measured with a benzoic acid (BA) chemical probe. As BA is lost and 3-hydrobenzoic acid (HBA) formed when the hydroxyl radical is scavenged, the hydroxyl radical concentration is calculated and the primary method of photolysis determined (e.g., indirect or direct). Benzoic acid was added to selected samples at a concentration of 6.4 mg/L. Loss of BA and production of HBA was measured using LC/MS. The concentration of the naphthenic acids was also measured using LC/MS.
Result	:	Naphthenic acid photolysis resulting from exposure to natural and artificial sunlight was limited. After one week of exposure to natural solar radiation, no individual compounds or mixtures were significantly degraded, although compositional changes were noted in the mixtures. Artificial solar radiation was similarly ineffective. Exposure to UV-245 radiation induced the most photolysis, but was only particularly effective on 4-MCHAA (half-life 3.2 – 3.6 hours) and was not an efficient means for complete removal of the other individual acids or complex mixtures from natural waters.

Remark	:	
Reliability	:	(1) Reliable with restrictions. Not a guideline study, but sufficiently documented to provide useful information.
Reference	:	McMartin, D.W., J.V. Headley, D.A. Friesen, K.M. Peru, and J.A. Gillies, 2004. Photolysis of naphthenic acids in natural surface water, Journal of Environmental Science and Health, Part A – Toxic/Hazardous Substances & Environmental Engineering, A39(6):1361-1383.

3.1.2 DISSOCIATION

Type	:	
Guideline/method	:	
pKa	:	
Year	:	
GLP	:	
Test substance	:	
Approx. water solubility	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Naphthenic acids exist as weak acids, with most pKa values being reported at about 5. At low pHs, they exist in their undissociated form and tend to partition onto solids. At high pHs, they exist in their dissociated form and become more mobile. (Appendix C)
Reliability	:	
Reference	:	

3.2.1 MONITORING DATA

Type of measurement	:	
Media	:	
Concentration	:	mg/l
Substance measured	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

3.3.1 TRANSPORT (FUGACITY)

Type	:	
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Year	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Using EPIWIN v3.10, Level I fugacity modeling was performed for a range of naphthenic acids covering the predominant molecular weight and ring

structures reported to predominate in Athabasca oil sands extracts. The principal distribution of these constituents following environmental release would be to soil and/or sediment, with overwhelming (98%) partitioning to soil. (Appendix C)

Reliability :
Reference :

3.5 BIODEGRADATION

Type :
Guideline/method : Non-guideline study
Inoculum : Sodium naphthenate-degrading enrichment cultures derived from oil sands tailings water.
Concentration : related to
related to
Contact time :
Degradation : (±) % after day(s)
Result :
Kinetic of test subst. : 50% converted to CO_2 in a 24-d period.
%
%
%
%
Control substance :
Kinetic : %
%
Deg. product :
Year : 1994
GLP :
Test substance : Commercial sodium naphthenate mixture
Deg. products CAS# :
Method :
Method detail :
Result : Commercial mixtures of the sodium salts of naphthenic acids were shown to degrade and mineralize to CO_2 when inoculated with microbial populations indigenous to oil sands tailings. Approximately 50% of the organic carbon was converted to CO_2 over a 24-d period. Three of four model naphthenic acid compounds were also degraded by the enrichment cultures, with approximately 40-50% of the organic carbon converted to CO_2 over a 24-d period.
Remark : Additional studies by Clemente et al. (2004) monitored the concentration and composition of naphthenic acids in aerobic biodegradation studies using sodium salts of naphthenic acids. Within 10 days of incubation with enrichment cultures on naphthenic acid-degraders, naphthenic acids concentration dropped from about 100 to <10 mg/L, accompanied by release of about 60% of the carbon as CO_2. GC/MS results indicated that the lower molecular weight acids (n = 5-13) were degraded more readily than high molecular weight acids. Clemente, J.S., M.D. Mackinnon, and P.M. Fedorak, 2004. Aerobic biodegradation of two commercial naphthenic acids preparations, Environ. Sci. Technol. 38:1009 – 1016.
Reliability :
Reference : Herman et al. 1994. Biodegradation of naphthenic acids by microbial populations indigenous to oil sands tailings. Can. J. Microbiol. 40:467-477; Appendix C

3.7 BIOCONCENTRATION

Type	:		
Guideline/method	:		
Species	:		
Exposure period	:	at	°C
Concentration	:		
BCF	:		
Elimination	:		
Year	:		
GLP	:		
Test substance	:		
Method	:		
Method detail	:		
Result	:		
Remark	:		
Reliability	:		
Reference	:		

4.1 ACUTE TOXICITY TO FISH

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	
NOEC	:	
LC0	:	
LC50	:	5.6 – 7.1 mg/L for bluegill
LC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Data in the U.S. EPA ECOTOX database from three references indicate an 96-h LC50 range for naphthenic acids of 5.6 – 7.1 mg/L for bluegill. The 96-h LC50 for another fish species, the zebra fish (*Danio rerio*), is reported as 16.3 mg/L for naphthenic acids. (U.S. Environmental Protection Agency. 2005. ECOTOX Database System. http://www.epa.gov/ecotox). Further information about these studies, and several additional references, is given in Appendix C. Commercial sodium salts of naphthenic acid produced LC50 values of 50 mg/L for kutum (*Rutulis frisii* kutum) and sturgeon (*Acipenser gueldenstaedi*) and 75 mg/L for roach (*Rutulis rutulis* caspicus) (Dokholyan and Magomedov, 1983, cited in Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355).
Reliability	:	
Reference	:	

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	
Guideline/method	:	
Species	:	
Exposure period	:	
NOEC	:	
EC0	:	
EC50	:	
EC100	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	

Result	:	
Remark	:	A 96-h LC50 of 4.8 mg/L for calcium naphthenate has been reported for the marine copepod, *Nitocra spinipes*. (Bengtsson, B.E. and M. Tarkpea. 1983. The acute aquatic toxicity of some substances carried by ships. Mar. Pollut. Bull. 14:213-214). The zooplankton species Nephargoides maeoticus tolerated naphthenic acids concentrations up to only 0.15 mg/L (Dokholyan and Magomedov, 1984, cited in Clemente, J.S. and P.M. Fedorak, 2005, A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere 60:585-600).
Reliability	:	
Reference	:	

4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Type	:	
Guideline/method	:	
Species	:	
Endpoint	:	
Exposure period	:	
NOEC	:	
LOEC	:	
EC0	:	
EC10	:	
EC50	:	
Other	:	
Other	:	
Other	:	
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	The toxicity of naphthenic acids to populations of the freshwater diatom, *Navicula seminulum*, has been measured. The 96-h EC50 for growth ranged from 26.0 – 80.5 mg/L (Academy of Natural Sciences. 1960. Cited in the EPA ECOTOX Database 2005. http://www.epa.gov/ecotox).
Reliability	:	
Reference	:	

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In vtro/in vivo	:	
Type	:	
Guideline/method	:	
Species	:	
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Route of administration	:	
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st.: 2nd.: 3rd.:
Toxic behavior	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.1.1 ACUTE ORAL TOXICITY

Type	:	Acute oral LD50
Guideline/Method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Male
Number of animals	:	5 per dose level (7 dose levels)
Vehicle	:	None - administered undiluted
Doses	:	1.0, 1.47, 2.15, 3.16, 4.64, 6.81, and 10 g/kg bw
LD50	:	5.88 g/kg bw (4.31 - 8.02 g/kg bw)
Year	:	1979
GLP	:	unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)
Method	:	
Method detail	:	Rats were observed at 1, 2, 4 and 6 hours after dosing and then daily for 14 days. Mortality, toxicity, and pharmacological effects were recorded. Body weights were recorded at pretest and in the survivors at 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross

pathology.

Result	:	Deaths occurred at dose levels of 3.16 g/kg and higher. Significant pre-death toxic signs included tremors, lethargy, ptosis, ataxia, prostration, negative righting reflex, flaccid muscle tone, piloerection, diarrhea, chromodacryorrhea, dyspnea and chromorhinorrhea. Body weight changes were noted in the survivors. Significant necropsy findings in the animals that died included dilated hearts and gastrointestinal irregularities.
Remark	:	Other data for rats includes an LD50 of 3.0 g/kg bw for naphthenic acid fraction from crude kerosene acids and 5.2 g/kg bw for naphthenic acid fraction from mixed crude oils (Rockhold, 1955, as cited in Appendix C). An oral acute toxicity test in rats with a mixture of naphthenic acids isolated from Athabasca oil sands produced appetite suppression, hepatoxicity and cardiovascular effects with a single dose of 300 mg/kg. (Rogers, V.V., et al., Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355). An LD50 of 3.55 g/kg for mice was reported by Pennisi and Lynch, 1977 (as cited in Appendix C).
Reliability	:	(1) Reliable without restrictions, as assessed in Appendix C
Reference	:	Exxon, 1979. Acute Oral Toxicity of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C.

5.1.2 ACUTE INHALATION TOXICITY

Type	:
Guideline/method	:
Species	:
Strain	:
Sex	:
Number of animals	:
Vehicle	:
Concentrations	:
Exposure time	:
LC50	:
Year	:
GLP	:
Test substance	:
Method	:
Method detail	:
Result	:
Remark	:
Reliability	:
Reference	:

5.1.3 ACUTE DERMAL TOXICITY

Type	:	Acute dermal LD50 with irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand White
Sex	:	Male and female
Number of animals	:	2 per sex
Vehicle	:	None – administered undiluted
Doses	:	3.16 g/kg
LD50	:	> 3.16 g/kg
Year	:	1979
GLP	:	Unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)

Method	:	
Method detail	:	The test substance was applied dermally to the clipped abraded abdomens of each animal. The area was covered with gauze and secured by a thick plastic binder, which was removed after 24 hours, and the skin washed with water or corn oil. Animals were then observed for mortality and toxic effects at 2 and 4 hours, and once daily thereafter. Body weight was recorded before and after the test. Dermal irritation was recorded at 1, 3, 7, 10 and 14 days. Mortality, toxicity and pharmacological effects were observed at 1, 2, 4, and 6 hours after dosing and once daily for 14 days. At 14 days the survivors were sacrificed. All animals were examined for gross pathology.
Result	:	No deaths occurred. Symptoms of toxicity appeared 2 to 4 hours after dosing and 3 out of 4 animals showed signs of toxicity until day 12 or 13. During the first five days, all animals displayed one or more of the following symptoms: lethargy, diarrhea, ptosis, adipsia, anorexia, and few feces. The test substance was judged to be moderately to severely irritating to the occluded skin. Mean values for erythema and edema at intact sites were 1.69 and 1.3, respectively.
Remark	:	
Reliability	:	(1) Reliable without restriction, as assessed in Appendix C
Reference	:	Exxon, 1979. Acute Dermal Toxicity of MRD-79-10 in Rabbits, MB 79-3702, as cited in Appendix C

5.2.1 SKIN IRRITATION

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
Classification	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	Moderately to severely irritating to rabbits.
Remark	:	See results of acute dermal LD50 study, described above.
Reliability	:	
Reference	:	

5.2.2 EYE IRRITATION

Type	:	Eye irritation
Guideline/method	:	
Species	:	Rabbit
Strain	:	New Zealand white
Sex	:	Male and female
Concentration	:	
Dose	:	
Exposure time	:	
Number of animals	:	3 per sex

Vehicle	:	None – administered undiluted
Classification	:	
Year	:	1979
GLP	:	Unknown
Test substance	:	MRD-79-10 (raw naphthenic acid derived from kerosene)
Method	:	
Method detail	:	0.1 mL of test substance was placed into the conjunctival sac of the eye of each of the six rabbits. The untreated eye served as a control. Animals were observed at 1 and 4 hours, and on days 1, 2, 3, 4 and 7. If a positive score was noted on day 7, ocular readings were scored on day 10. If an positive score was noted on day 10, observations were made on day 14. Fluorescein was used in examining ocular reactions on day 3 and after. The Draize technique was used as the scoring system.
Result	:	One animal had a positive corneal score on days 1 and 2; one animal had a positive iris score at hours 1 and 4. All animals exhibited positive conjunctival scores at some point during the first three days of observation. By day 4, no animals showed positive scores. The test material was judged to be an irritant. In a later summary report, eye irritation was judged to be moderate.
Remark	:	
Reliability	:	(1) Reliable without restrictions, as assessed in Appendix C
Reference	:	Exxon, 1979. Eye Irritation Study of MRD-79-10 in Rats, MB 79-3702, as cited in Appendix C

5.4 REPEATED DOSE TOXICITY

Type	:	Oral 90-d subchronic toxicity test
Guideline/method	:	
Species	:	Rat
Strain	:	Wistar
Sex	:	Female
Number of animals	:	12 per dose level
Route of admin.	:	Oral gavage
Exposure period	:	
Frequency of treatment	:	1 dose/day, 5 days/week
Post exposure period	:	
Dose	:	0.6, 6, or 60 mg/kg bw (aqueous solutions of naphthenic acids)
Control group	:	Yes (7 ml tap water)
NOAEL	:	6 mg/kg/day
LOAEL	:	60 mg/kg/day (5 doses per week)
Other	:	
Year	:	2002
GLP	:	Unknown
Test substance	:	Mixture of naphthenic acids (acyclic and 1-, 2-, 3-, and 4-ringed compounds, administered as sodium salt solutions) isolated from tailings pond water from Athabasca oil sands
Method	:	
Method detail	:	Animals were monitored daily. Changes in body weight, food consumption and behavioral or clinical signs recorded. Blood samples were collected from the ventral tail vein on day 45 of dosing and analyzed for plasma biochemical and hematological effects. Blood samples were similarly analyzed from cardiac punctures on day 91. Following euthanization, the liver, kidney, spleen, heart, lung and ovaries were examined.
Result	:	Significant physical, clinical, and pathological changes at a dose level of 60 mg/kg/day (5 doses per week). No significant adverse effects were seen at a dose level of 6 mg/kg/day. Several parameters suggested that the liver

		was the primary target organ in this study. Liver weight was increased 35% above control values in the high dose group. Body weight gain was also reduced 8-9% in this exposure group compared to controls. Plasma cholesterol was reduced and amaylase activity increased in the high dose group.
Remark	:	
Reliability	:	(2) Reliable with restriction. Only female rats were used and a limited number of organs examined.
Reference	:	Rogers et al. 2002. Acute and subchronic toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66:347-355.

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Mutagenicity
Guideline/method	:	Ames assay
System of testing	:	Bacteria *in vitro*
Species	:	*Salmonella typhimurium*
Strain	:	TA100, TA1535, TA97, TA98
Test concentrations	:	1 – 1000 ug/L depending upon strain
Cytotoxic concentr.	:	
Metabolic activation	:	With and without
Year	:	1993
GLP	:	Yes
Test substance	:	Calcium naphthenate
Method	:	
Method detail	:	Activation was with induced male Sprague Dawley rat liver S9 and induced male Syrian hamster liver S9.
Result	:	
Remark	:	Sodium naphthenate was also negative in the *Salmonella* mutagenicity test, performed similarly.
Reliability	:	(1) Reliable without restriction
Reference	:	Study ID A21560 and Study ID 278018, National Toxicology Program (http://ntp-server.niehs.nih.gov)

Type	:	In vitro cytogenetics
Guideline/method	:	
System of testing	:	
Species	:	
Strain	:	
Test concentrations	:	
Cytotoxic concentr.	:	
Metabolic activation	:	
Year	:	
GLP	:	Yes
Test substance	:	Sodium naphthenate
Method	:	
Method detail	:	
Result	:	Negative results were obtained for chromosome aberrations, while positive results were obtained for Sister Chromatid Exchanges.
Remark	:	
Reliability	:	(1) Reliable without restriction
Reference	:	Study ID 058122, National Toxicology Program (http://ntp-server.niehs.nih.gov)

Type	:	Mutagenicity
Guideline/method	:	

System of testing	:	
Species	:	Mouse Lymphoma
Strain	:	L5178Y (TK+/TK-)
Test concentrations	:	0.005 – 0.037 UL/ML
Cytotoxic concentr.	:	
Metabolic activation	:	none
Year	:	
GLP	:	
Test substance	:	Naphthenic acid, calcium salt (61789-36-4)
Method	:	Suspension Plate
Method detail	:	
Result	:	Positive
Remark	:	
Reliability	:	
Reference	:	CCRIS (Record # 1169) ()

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:
Guideline/method	:
Species	:
Strain	:
Sex	:
Route of admin.	:
Exposure period	:
Doses	:
Year	:
GLP	:
Test substance	:
Method	:
Method detail	:
Result	:
Remark	:
Reliability	:
Reference	:

5.8.2 DEVELOPMENTAL TOXICITY

Type	:
Guideline/method	:
Species	:
Strain	:
Sex	:
Route of admin.	:
Exposure period	:
Frequency of treatment	:
Duration of test	:
Doses	:
Control group	:
NOAEL maternal tox.	:
NOAEL teratogen.	:
Other	:
Other	:
Other	:
Year	:
GLP	:

Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

5.8.3 TOXICITY TO REPRODUCTION

Type	:	Dermal exposure
Guideline/method	:	
In vitro/in vivo	:	In vivo
Species	:	12 male New Zealand White rabbits
Strain	:	
Sex	:	Male
Route of admin.	:	Dermal
Exposure period	:	6 hours/day
Frequency of treatment	:	5 days/week
Duration of test	:	
Doses	:	2 ml undiluted material
Control group	:	12 male
Year	:	1984
GLP	:	
Test substance	:	An over-based calcium naphthenate in mineral oil
Method	:	
Method detail	:	
Results	:	Results of the oral reproduction study are consistent with a one generation dermal reproduction study in male rabbits conducted on SAP 011, an overbased calcium naphthenate in mineral oil. A group of 12 male New Zealand White rabbits was dermally exposed to 2 ml of undiluted SAP 011 for 6 hours daily for 5 days each week over a 10-week period. Following the exposure period, each male rabbit was mated with two untreated female rabbits. Males were subsequently necropsied and their reproductive tracts examined macroscopically and microscopically. Female rabbits were necropsied on day 29 of gestation and examined for reproductive parameters. Study results showed no adverse effects on reproductive performance due to male exposure. There were no adverse signs of toxicity either systemically or at the site of application in treated males, as well as no pathological findings of the reproductive tract that could be related to SAP 011 exposure.
Remark	:	
Reliability	:	(2) Reliable with restrictions
Reference	:	Dix, K.M. and S.L. Cassidy. 1983. Toxicity studies on oil additives: one generation reproduction study in male rabbits repeatedly treated dermally with SAP 0111 for 10 weeks. External Report SBER.84.002. Shell Research Ltd. (NTIS No. OTS0507494)

6.0 OTHER INFORMATION

6.1 Carcinogenicity

In a study in which calcium naphthenate was dermally administered to female mice (two times per day for two years), twelve epidermal and one dermal tumor at the treated sites were observed in eight of the exposed mice.

Four of the tumors were malignant and none were benign. The first of these neoplasms were reported after 392 days of treatment. No metastatic tumors were present. (Appendix C)

6.2 Skin sensitization

Appendix C: Zinc Chloride

1.0 SUBSTANCE INFORMATION

Generic Name	:	Zinc chloride
Chemical Name	:	Zinc dichloride
CAS Registry No.	:	7646-85-7
Component CAS Nos.	:	
EINECS No.	:	231-592-0
Structural Formula	:	$ZnCl_2$
Additional description	:	
Molecular Weight	:	136.29
Synonyms and Tradenames	:	Zinc (II) chloride; Butter of zinc; zinc butter; RTECS ZH1400000
References	:	ATSDR, 2003 (Agency for Toxic Substances and Disease Registry, Draft Toxicological Profile for Zinc, September 2003)

2.1 MELTING POINT

Type	:	
Guideline/method	:	
Value	:	290 °C
Decomposition	:	
Sublimation	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ.

2.2 BOILING POINT

Type	:	
Guideline/method	:	
Value	:	732 °C
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ.

2.3 DENSITY

Type	:	
Guideline/method	:	
Value	:	2.907 at 25°C
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ.

2.4 VAPOR PRESSURE

Type	:	
Guideline/method	:	
Value	:	
Decomposition	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Expected to be very low based on melting point and boiling point data.
Reliability	:	
Reference	:	

2.5 PARTITION COEFFICIENT

Type	:	
Guideline/method	:	
Partition coefficient	:	
Log Pow	:	
pH value	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Not applicable – compound dissociates and ionizes in water
Reliability	:	
Reference	:	

2.6.1 SOLUBILITY IN WATER

Type	:	
Guideline/method	:	
Value	:	4.32 X 10^6 mg/L at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
PKa	:	at °C
Description	:	
Stable	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	2 (reliable with restrictions): Source is well established data compendium.
Reference	:	O'Neil, M.J., Smith, A., Heckelman, P.E., and J.R. Obenchain (eds.). 2002. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Ed. Merck & Co., Inc., Whitehouse Station, NJ.

2.7 FLASH POINT

Type	:	
Guideline/method	:	
Value	:	Not flammable
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

3.1.1 PHOTODEGRADATION

Type		
Guideline/method	:	
Light source	:	
Light spectrum	:	
Relative intensity	:	based on
Spectrum of substance	:	lambda (max, >295nm) :
		epsilon (max) :
		epsilon (295) :
Conc. of substance	:	at °C
DIRECT PHOTOLYSIS		
Halflife (t1/2)	:	
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	
Degradation	:	
Deg. product	:	
Year	:	
GLP	:	
Test substance	:	
Deg. products CAS#	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Not applicable – the metal will not degrade
Reliability	:	
Reference	:	

3.2.1 MONITORING DATA

Type of measurement	:	
Media	:	
Concentration	:	mg/l
Substance measured	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	
Reliability	:	
Reference	:	

3.3.1 TRANSPORT (FUGACITY)

Type	:	
Media	:	
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Year	:	
Test substance	:	

Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

3.5 BIODEGRADATION

Type :
Guideline/method :
Inoculum :
Concentration : related to
related to
Contact time :
Degradation : (±) % after day(s)
Result :
Kinetic of test subst. : % (specify time and % degradation)
%
%
%
%
Control substance :
Kinetic : %
%
Deg. product :
Year :
GLP :
Test substance :
Deg. products CAS# :
Method :
Method detail :
Result :
Remark : Not applicable – the metal will not degrade
Reliability :
Reference :

3.7 BIOCONCENTRATION

Type :
Guideline/method :
Species :
Exposure period : at °C
Concentration :
BCF :
Elimination :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

4.1 ACUTE TOXICITY TO FISH

Type	:	Acute
Guideline/method	:	Flow-through, freshwater
Species	:	Rainbow trout (*Onchorhynchus mykiss*)
Exposure period	:	96 hr
NOEC	:	
LC0	:	
LC50	:	93 – 0.815 µg Zn/L (depending on juvenile life-stage)
LC100	:	
Limit test	:	
Analytical monitoring	:	No
Year	:	1978
GLP	:	No
Test substance	:	Zinc chloride
Method	:	
Method detail	:	The toxicity of zinc chloride to four juvenile stages of rainbow trout (alvins, swim-up fry, parr, smolts) was determined in 96-h flow-through tests.
Result	:	LC50 values varied by life stage with the swim-up fry being the most sensitive.
Remark	:	The bioavailability and resultant aquatic toxicity of zinc chloride is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature. Reported 96-h LC50 values for zinc chloride (expressed as zinc) for various species of fish include 0.29 mg Zn/L and 0.42 mg Zn/L for bluegill (*Lepomis macrochirus*); 0.093 – 2.17 mg Zn/L for rainbow trout (*Onchorhynchus mykiss*), 0.45 - 2.25 mg Zn/L for common mirror-colored carp (*Cyprinus carpio*) and 1.70 mg Zn/L for sheepshead minnow (*Cyprinodon variegatus*) (U.S. EPA, ECOTOX database, 2003).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Chapman, G.A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of Chinook and steelheads. Trans. Am. Fish. Soc., 107(6):841-847.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Acute
Guideline/method	:	Flow-through, freshwater
Species	:	*Daphnia magna*
Exposure period	:	48 hr
NOEC	:	
EC0	:	
EC50	:	799 µg Zn/L
EC100	:	
Limit test	:	
Analytical monitoring	:	
Year	:	1982
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Flow-through
Method detail	:	
Result	:	
Remark	:	The bioavailability and resultant aquatic toxicity of zinc chloride is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature. Reported 48-h EC50 values for zinc chloride (expressed as zinc) for *Daphnia magna* include 0.33, 0.52, 0.66 and 0.80

		mg Zn/L (U.S. EPA, ECOTOX database, 2003). For several crustaceans, including *Daphnia magna*, *Ceriodaphnia dubia*, and *Ceriodaphnia reticulata*, reported 48-h EC50 values ranged from 0.068 to 0.86 mg Zn/L, for zinc tested as zinc chloride or zinc sulfate.
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Attar, E.N. and E.J. Maly. 1982. Acute toxicity of cadmium, zinc, and cadmium-zinc mixtures to *Daphnia magna*. Arch. Environ. Contam. Toxicol., 11(3):291-296.

4.3 TOXICITY TO AQUATIC PLANTS (E.G., ALGAE)

Type	:	Algal growth assay
Guideline/method	:	Static
Species	:	*Selenastrum capricornutum*
Endpoint	:	Growth
Exposure period	:	96 hr
NOEC	:	
LOEC	:	
EC0	:	
EC10	:	
EC50	:	44.7 µg Zn/L
Limit test	:	
Analytical monitoring	:	
Year	:	
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Microplate algal assay
Method detail	:	
Result	:	
Remark	:	The bioavailability and resultant aquatic toxicity of zinc is affected by a variety of factors, including water hardness, pH, dissolved organic carbon and temperature The reported 72-h EC50 for the marine diatom *Skeletonema costatum* was 0.142 mg Zn/L (U.S. EPA, ECOTOX database, 2003).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Alaise, C., R. Legault, N. Bermingham, R. Van Coillie, and P. Vasseur. 1986. A simple microplate algal assay technique for aquatic toxicity assessment. Toxic. Assess., 1:261-281.

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In vitro/in vivo	:	
Type	:	
Guideline/method	:	
Species	:	
Number of animals	:	
Males	:	
Females	:	
Doses	:	
Males	:	
Females	:	
Vehicle	:	
Route of administration	:	
Exposure time	:	
Product type guidance	:	
Decision on results on acute tox. tests	:	
Adverse effects on prolonged exposure	:	
Half-lives	:	1st.: 2nd.: 3rd.:
Toxic behavior	:	
Deg. product	:	
Deg. products CAS#	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Zinc is an essential element in nutrition, and is important in membrane stability, in over 300 enzymes, and in the metabolism of proteins and acids. (WHO, 2001, Environmental Health Criteria 221, Zinc). Absorption of zinc in laboratory animals can vary from 10-40% depending upon nutritional status and other ligands in the diet. Absorbed zinc is mainly deposited in muscle, bone, liver, pancreas, kidney and other organs. The biological half-life of zinc ranges from 4 to 50 days in rats depending on the administered dose (WHO, 2001, Environmental Health Criteria 221, Zinc). Increases in zinc concentration in the bodies of experimental animals exposed to zinc are accompanied by reduced levels of copper, suggesting that some of the signs of toxicity ascribed to zinc may be caused by zinc-induced copper deficiency. Moreover, studies have shown that exposure to zinc alters the levels of other essential metals, including iron. Zinc deficiency in animals is characterized by a reduction in growth and cell replication, adverse reproductive and developmental effects, and reduced immunoresponsiveness. (WHO, 2001, Environmental Health Criteria 221, Zinc).
Reliability	:	
Reference	:	

5.1.1 ACUTE ORAL TOXICITY

Type	:	Oral
Guideline	:	Not specified
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male
Number of animals	:	10 per dose group
Vehicle	:	Water
Doses	:	Not specified
LD50	:	1,100 mg/kg b.w. as $ZnCl_2$ (95% C.I. = 661 – 1,830 mg/kg b.w.) 528 mg/kg b.w. as zinc (95% C.I. = 316 – 875 mg/kg b.w.)
Year	:	1988
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Single doses administered intragastrically.
Method detail	:	Rats weighed 230 – 280 g. Solution concentrations were adjusted so that a 300–g rat received a 1 ml dose. Solutions were adjusted to a pH of between 6.0 and 7.0, using sodium biocarbonate when necessary.
Result	:	Acute LD50 values of zinc chloride were also determined using i.p administration in this study. The toxicity of zinc chloride to rats was much greater after i.p. administration with an LD50 of 58 mg/kg b.w. when expressed as $ZnCl_2$ (95% C.I. = 43 – 79) or 28 mg/kg b.w. when expressed as zinc (95% C.I. = 21 – 38). The much lower toxicity by the oral route of administration suggests a low rate of absorption of zinc chloride from the gastrointestinal tract.
Remark	:	Acute oral toxicity in rodents exposed to zinc compounds is low, and the level at which zinc produces no adverse effect in rats is approximately 160 mg/kg body weight (WHO, 2001, Environmental Health Criteria 221, Zinc). Of the compounds zinc nitrate, zinc sulfate, zinc chloride and zinc acetate, zinc acetate was the most toxic, with oral LD50 values of 237 mg Zn/kg bw (rat) and 86 mg Zn/kg bw (mouse).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Domingo, J.L., J.M. Llobet, J.l. Paternain, and J. Corbella. 1988. Acute zinc intoxication: comparison of the antidotal efficacy of several chelating agents. Vet. Hum. Toxicol., 30(3): 224-228.

Type	:	Oral
Guideline/Method	:	Not specified
Species	:	Mouse
Strain	:	Swiss
Sex	:	Male
Number of animals	:	10 per dose group
Vehicle	:	Water
Doses	:	Not specified
LD50	:	1,260 mg/kg b.w. as $ZnCl_2$ (95% C.I. = 775 – 2,300 mg/kg b.w.) 605 mg/kg b.w. as zinc (95% C.I. = 370 – 1,099 mg/kg b.w.)
Year	:	1988
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Single doses administered intragastrically.
Method detail	:	Mice weighed 24 – 28 g. Solution concentrations were adjusted so that a 30–g mouse received a 0.21 ml dose. Solutions were adjusted to a pH of between 6.0 and 7.0, using sodium biocarbonate when necessary.
Result	:	Acute LD50 values of zinc chloride were also determined using i.p administration in this study. The toxicity of zinc chloride to mice was much greater after i.p. administration with an LD50 of 91 mg/kg b.w. when

		expressed as $ZnCl_2$ (95% C.I. = 57 – 146) or 44 mg/kg b.w. when expressed as zinc (95% C.I. = 27 – 69). The much lower toxicity by the oral route of administration suggests a low rate of absorption of zinc chloride from the gastrointestinal tract.
Remark	:	
Reliability	:	2, reliable with restrictions: Comparable to guideline study with adequate documentation.
Reference	:	Domingo, J.L., J.M. Llobet, J.l. Paternain, and J. Corbella. 1988. Acute zinc intoxication: comparison of the antidotal efficacy of several chelating agents. Vet. Hum. Toxicol., 30(3): 224-228.

5.1.2 ACUTE INHALATION TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Concentrations	:	
Exposure time	:	
LC50	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Zinc chloride is a primary ingredient in smoke bombs, resulting in respiratory injury. In a 10-minute inhalation study with rats, zinc chloride aerosol was lethal at concentrations as low as 940 mg Zn/m^3 (Risk Assessment for Zinc Metal, 2001, draft).
Reliability	:	
Reference	:	

5.1.3 ACUTE DERMAL TOXICITY

Type	:	
Guideline/method	:	
Species	:	
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
LD50	:	
Year	:	
GLP	:	
Test substance	:	
Method	:	
Method detail	:	
Result	:	
Remark	:	Zinc chloride is reported to cause moderate to severe skin irritation in the rabbit, guinea pig and mouse at 0.48 mg Zn/cm2 while zinc acetate at 7.2

mg Zn/cm^2 was reported to be irritating to the rabbit and mouse but caused no effects in the guinea pig (ATSDR, 1994, Toxicological Profile for Zinc).

Reliability :
Reference :

5.2.1 SKIN IRRITATION

Type :
Guideline/method :
Species :
Strain :
Sex :
Concentration :
Exposure :
Exposure time :
Number of animals :
Vehicle :
Classification :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark : Zinc chloride, applied daily as a 1% aqueous solution in an open patch test for 5 days, was severely irritant in rabbits, guinea pigs and mice, inducing epidermal hyperplasia and ulceration. (Lansdown, 1991 as cited in WHO, 2001, Environmental Health Criteria 221, Zinc).
Reliability :
Reference :

5.2.2 EYE IRRITATION

Type :
Guideline/method :
Species :
Strain :
Sex :
Concentration :
Dose :
Exposure time :
Number of animals :
Vehicle :
Classification :
Year :
GLP :
Test substance :
Method :
Method detail :
Result :
Remark :
Reliability :
Reference :

5.4 REPEATED DOSE TOXICITY

Type	:	28-d Oral
Guideline	:	Not specified
Species	:	Rat
Strain	:	Wistar
Sex	:	Both male and female
Number of animals	:	13 males; 17 females in treatment group
Route of admin.	:	Drinking water
Exposure period	:	4 weeks
Frequency of treatment	:	Continuous
Post exposure period	:	None
Doses	:	11.66 mg Zn/kg b.w./day in males and 12.75 mg Zn/kg b.w./day in females on average from 0.12 mg Zn/cm^3 in water
Control group	:	Yes
NOAEL	:	None
LOAEL	:	12 mg Zn/kg b.w./day
Other	:	
Year	:	1992
GLP	:	No
Test substance	:	Zinc chloride
Method	:	
Method detail	:	Two-month-old Wistar rats of both sexes received zinc chloride in their drinking water for a period of 4 weeks. Liquid consumption was monitored so that the average daily Zn exposure could be calculated. At study termination, rats were weighed, bled, and sacrificed. Hematological indices were determined on blood samples.
Result	:	Zinc treatment had no effect on the survival or body weight gain of exposed rats. Zinc treatment also had no appreciable affect on the composition of bone marrow cells. However, erythrocytes counts and hemoglobin levels in the peripheral blood were significantly decreased in Zn-exposed males and females compared to controls, while the numbers of leukocytes, neutrophils, and lymphocytes in male rats were increased compared to controls.
Remark	:	Long-term oral exposure to zinc compounds indicates the target organs of toxicity to be the hematopoeitic system in rats, ferrets and rabbits; the kidney in rats and ferrets; and the pancreas in mice and ferrets (WHO, 2001, Environmental Health Criteria 221, Zinc). Zinc acetate given to rats in water over three months yielded NOAEL values of 95 to 191 mg Zn/kg/d. During a 13-week exposure to zinc sulfate via the diet, NOAEL values for the rat ranged from 53 to 565 mg Zn/kg/day and for the mouse were 104 mg Zn/kg/d, based upon various parameters. (ATSDR, 2003, Draft Toxicological Profile for Zinc).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Zaporowska, H. and W. Wasilewski. 1992. Combined effect of vanadium and zinc on certain selected haematological indices in rats. Comp. Biochem. Physiol., 103C: 143-147.

Type	:	13-week Oral
Guideline/method	:	Not specified
Species	:	Rat
Strain	:	Wistar
Sex	:	Male and female
Number of animals	:	12 of each sex per treatment group
Route of admin.	:	Diet
Exposure period	:	13 wk
Frequency of treatment	:	Continuous

Post exposure period	:	None
Doses	:	0, 300, 3,000, or 30,000 ppm in diet (equivalent to an average daily intake of 23.2, 234, or 2,514 mg $ZnSO_4$/kg/d in males and 24.5, 243, or 2,486 mg $ZnSO_4$/kg/d in females
Control group	:	Yes, for both males and females
NOAEL	:	3,000 ppm in diet (equivalent to approximately 234 mg $ZnSO_4$/kg/d in males and 243 mg $ZnSO_4$/kg/d in females)
LOAEL	:	30,000 ppm in diet (equivalent to approximately 2,514 mg $ZnSO_4$/kg/d in males and 2,486 mg $ZnSO_4$/kg/d in females)
Other	:	
Year	:	1981
GLP	:	No
Test substance	:	$ZnSO_4 \cdot 7H_2O$
Method	:	
Method detail	:	Groups of male and female rats (12 each) were feed diets containing zinc sulfate for 13 weeks. Animals were observed daily for clinical signs of toxicity and weighed weekly. Feed and water intake was measured twice per week. Prior to study termination, blood samples were collected and analyzed for hematological and biochemical parameters. Following necropsy, gross pathological and histopathological examinations were conducted on selected target organs and tissues. Organs weights were also determined.
Results	:	No compound-related mortality was observed at any dose level. The only clinical signs of toxicity were behavioral (removal of chow from the feeding container) and confined to the highest feeding level (30,000 ppm). At the highest dose level, food consumption, water intake and growth were reduced, particularly in males. A moderate reduction in the total leukocyte count was observed in both sexes in the high dose groups, whereas males in this group also showed slightly decreased hematocrit and hemoglobin levels. GOT and GPT concentrations were decreased in all male groups but there was no dose-response trend. Total protein, cholesterol and calcium in the blood were decreased in high dose males, whereas only calcium was elevated in high dose females. Necropsy results indicated no remarkable gross lesions in rats at any dose level, although the weights (both absolute and relative) of the livers and kidneys of the males in the 30,00 ppm group showed a slight to moderate decrease. Histopathological examinations showed pancreatic lesions attributable to treatment in the high dose groups. Lesions consisted of degeneration and necrosis of the acinar cells, clarification of centroacinar cells, and interstitial fibrosis.
Remark	:	While not conducted on the zinc chloride salt, the results of this study on hydrated zinc sulfate are considered relevant for assessing the potential hazard of the chloride because both salts are soluble and expected to have a similar bioavailability and toxicity. In general, after oral or dermal exposure, the toxicities of all zinc compounds are comparable (ATSDR, 2003. Draft Toxicological Profile for Zinc).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Maita, K., M. Hirano, K. Mitsumori, K. Takahashi, and Y. Shirasu. 1981. Subacute toxicity studies with zinc sulfate in mice and rats. J. Pesticide Sci., 6: 327-336.
Type	:	13-week Oral
Guideline/method	:	Not specified
Species	:	Mouse
Strain	:	ICR (specific pathogen-free)
Sex	:	Male and female
Number of animals	:	12 of each sex per treatment group

Route of admin.	:	Diet
Exposure period	:	13 wk
Frequency of treatment	:	Continuous
Post exposure period	:	None
Doses	:	0, 300, 3,000, or 30,000 ppm in diet (equivalent to an average daily intake of 42.7, 458, or 4,927 mg $ZnSO_4$/kg/d in males and 46.4, 479, or 4,878 mg $ZnSO_4$/kg/d in females
Control group	:	Yes, for both males and females
NOAEL	:	3,000 ppm in diet (equivalent to approximately 458 mg $ZnSO_4$/kg/d in males and 479 mg $ZnSO_4$/kg/d in females)
LOAEL	:	30,000 ppm in diet (equivalent to approximately 4,927 mg $ZnSO_4$/kg/d in males and 4,878 mg $ZnSO_4$/kg/d in females)
Other	:	
Year	:	1981
GLP	:	No
Test substance	:	$ZnSO_4 \bullet 7H_2O$
Method	:	
Method detail	:	Groups of male and female mice (12 each) were feed diets containing zinc sulfate for 13 weeks. Animals were observed daily for clinical signs of toxicity and weighed weekly. Feed and water intake was measured twice per week. Prior to study termination, blood samples were collected and analyzed for hematological and biochemical parameters. Following necropsy, gross pathological and histopathological examinations were conducted on selected target organs and tissues. Organs weights were also determined.
Results	:	Although there were no obvious clinical signs of toxicity, four of 12 males in the high dose (30,000 ppm) group died or were killed *in extremis*. One female fed at this level also died. Histological findings in these animals revealed impairment of the urinary tract and regressive changes in the exocrine gland of the pancreas. Food consumption, water intake, and growth were depressed in the high dose groups, with the greatest effects seen in males. Male and female mice in the 30,000 ppm group showed moderately reduced levels of hematocrit and hemoglobin compared to controls; the leukocyte counts in these males were also decreased moderately. Mice of both sexes in the high dose groups showed a slight to moderate decrease in total protein, glucose and cholesterol, and a moderate to marked increase in alkaline phosphatase and urea nitrogen. Additional findings included depressed GPT levels in females, increased blood calcium levels in females, and increased GOT levels in males. Gross pathological changes in the high-dose animals included marked emaciation, ischemic discoloration of the kidney and thyroid, atrophy of the pancreas, edematous thickening of the upper small intestine, slight splenomegaly, and ulcers of the fore-stomach. Histopathological lesions were observed in the pancreas (swollen nuclei, necrosis of acinar cells), upper intestine (proliferation of epithelial cells), fore-stomach (ulcerations), spleen (proliferation of erythropoietic immature cells), and kidney (regression of renal cortex in females).
Remark	:	Results were consistent with those in rats (see previous robust summary); however, the effects on mice were generally more severe at the same level (ppm) in the diet. Most likely this was due to the much higher dose levels of zinc sulfate in mice compared to rats (approximately double on a mg/kg/d basis) due to their smaller size and greater relative food intake.
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Maita, K., M. Hirano, K. Mitsumori, K. Takahashi, and Y. Shirasu. 1981. Subacute toxicity studies with zinc sulfate in mice and rats. . J. Pesticide Sci., 6: 327-336.

5.5 GENETIC TOXICITY - MUTAGENICITY

Type	:	Mutagenicity
Guideline/method	:	Rec-assay
System of testing	:	Bacteria *in vitro*
Species	:	*Bacillus subtilis*
Strain	:	H17 (rec+) and M45 (rec-)
Test concentrations	:	0.05 M
Cytotoxic concentr.	:	Not determined
Metabolic activation	:	No
Year	:	1975
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Kada et al., 1972. Mutation Res., 16:165-174.
Method detail	:	An 0.05 ml aliquot of a 0.05 M zinc chloride solution was tested.
Result	:	At the concentration tested, there was no inhibition of either the rec+ or rec- strain of *Bacillus subtilis*, suggesting that zinc chloride did not cause DNA damage.
Remark	:	In 11 separate in vitro studies with zinc chloride or zinc sulfate, negative results were reported with the exception of two ambiguous results and one weakly positive result. (Risk Assessment for Zinc Metal, 2001, draft). Genotoxicity studies in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenicity following zinc exposure (ATSDR, 2003 Draft Toxicological Profile for Zinc). The results of short-term genotoxicity assays for zinc are equivocal. Responses in mutagenicity assays are thought to depend on the form (e.g., inorganic or organic salt) of the zinc tested (U.S. EPA, 2003, Integrated Risk Information System (IRIS) Summary for Zinc and Compounds).
Reliability	:	2 (reliable with restrictions): Acceptable study with adequate documentation.
Reference	:	Nisioka, H. 1975. Mutagenic activities of metal compounds in bacteria. Mutation Res., 31: 185-189.

Type	:	Mutagenicity
Guideline/method	:	Microscreen assay
System of testing	:	Bacteria *in vitro*
Species	:	*Escherichia coli*
Strain	:	$WP_s(\lambda)$
Test concentrations	:	Not specified
Cytotoxic concentr.	:	>1 mM
Metabolic activation	:	No
Year	:	1987
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Rossman et al., 1984. Environ. Mut., 6:59.
Method detail	:	
Result	:	Negative for Trp+ reversion, λ Prophage induction and WP2 comutagenenesis
Remark	:	
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Rossman, T.G., J.T. Zelikoff, S. Agarwal, and T.J. Kneip. 1987. Genetic toxicology of metal compounds: an examination of appropriate cellular

models. Toxicol. Environ. Chem., 14:251-262.

Type	:	Mutagenicity
Guideline/method	:	L5178Y/TK somatic cell point mutation assay
System of testing	:	Cultured mouse lymphoma cells – *in vitro*
Species	:	Mouse
Strain	:	L5178/TK$^{+/-}$
Test concentrations	:	1.21 – 12.13 µg/ml
Cytotoxic concentr.	:	Not determined
Metabolic activation	:	No
Year	:	1980
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Clive et al., 1972. Mutation Res., 16:77-87.
Method detail	:	
Result	:	Zinc chloride was not mutagenic under the test conditions.
Remark	:	
Reliability	:	2 (reliable with restrictions): Acceptable study with adequate documentation.
Reference	:	Amacher, D.E. and S.C. Paillet. 1980. Induction of trifluorothymidine-resistant mutants by metal ions in L5178Y/TK+/- cells. Mutation Res., 78: 279-288.

5.6 GENETIC TOXICITY - CLASTOGENICITY

Type	:	Chromosomal aberrations in bone marrow cells
Guideline/method	:	*In vivo*
Species	:	Mouse
Strain	:	C57B1
Sex	:	Male
Route of admin.	:	Diet
Exposure period	:	One month
Doses	:	0.5% Zn in feed
Year	:	1979
GLP	:	No
Test substance	:	Zinc chloride
Method	:	
Method detail	:	8-week-old mice kept on a normal (1.1% calcium) or low-calcium (0.03%) diet were exposed for one month to zinc chloride (0.5% Zn). After test termination, the bone marrow cells (50 metaphases/animal) from 10 animals were assayed for chromosomal aberrations.
Result	:	The body weights of mice fed zinc in the diet, either with normal or low calcium, were significantly reduced compared to their respective controls. Zinc treatment caused a significant increase in cells with structural aberrations (primarily dicentric chromosomes) for mice on low calcium diets. Aberrations were also increased in Zn-treated mice with normal calcium diets, but the increase was not statistically significant.
Remark	:	Studies on the induction of chromosome aberrations in bone marrow cells harvested from animals exposed to zinc compounds have yielded equivocal results. Increased aberrations have been seen in rats after oral exposure to zinc chloride in water (249 mg/L for 14 days) and in mice given intraperitoneal injections of zinc chloride (2-5 mg/kg as zinc chloride). In contrast, other studies have produced negative findings or have suggested that the induction of aberrations is contingent upon concomitant calcium deficiency. Negative results have been reported in the mouse micronucleus test (i.p. injection of zinc sulfate) and in the dominant lethal mutation assay

with mice (i.p. injection of zinc chloride at 15 mg/kg). (WHO, 2001, Environmental Health Criteria 221, Zinc).

Reliability : 2 (reliable with restrictions): Acceptable study with adequate documentation.

Reference : G. Deknudt and G.B. Gerber. 1979. chromosomal aberrations in bone-marrow cells of mice given a normal or a calcium-deficient diet supplemented with various heavy metals. Mutation Res., 68:163-168.

5.8.2 DEVELOPMENTAL TOXICITY

Type	:	Teratogenicity
Guideline	:	Not specified
Species	:	Mouse
Strain	:	CF-1 albino
Sex	:	Female
Route of admin.	:	Intraperitoneal
Exposure period	:	Day 8, 9, 10, or 11 of gestation
Frequency of treatment	:	Single dose
Duration of test	:	To gestation Day 18
Doses	:	12.5, 20.5, or 25 mg $ZnCl_2$/kg
Control group	:	Yes (distilled water only)
NOAEL maternal tox.	:	12.5 mg $ZnCl_2$/kg
NOAEL teratogen.	:	12.5 mg $ZnCl_2$/kg
Other	:	
Other	:	
Other	:	
Year	:	1977
GLP	:	No
Test substance	:	Zinc chloride
Method	:	
Method detail	:	Gravid female mice were given an i.p. injection of either 12.5, 20.5 or 25 mg $ZnCl_2$/kg on Day 8, 9, 10, or 11 of gestation. Following the respective treatments, the mice were allowed to continue their gestation uninterrupted until Day 18 (one day prior to expected delivery), when each pregnant mouse was sacrificed. The number of fetuses and resorption sites (metrial glands) was determined and recorded. Each fetus was then weighed, sexed, and examined for external defects. Every other fetus was processed for skeletal examination by the method of Staples and Schnell (1964).
Result	:	Zinc chloride, when administered in doses of 20.5 and 25 mg/kg, produced significant incidences of skeletal defects in fetuses as compared to those observed in the water-treated group on Day 11. Both doses also resulted in mortality of gravid females. The majority of defects involved the rib cage and included a ripple rib anomaly; however, the zinc salt failed to produce a significant incidence of soft tissue anomalies with either treatment regimen. As the dosage of $ZnCl_2$ was reduced, maternal and fetal toxicity, relative fetal weights, and the incidences of skeletal anomalies were correspondingly decreased. Maternal toxicity and incidences of skeletal anomalies were greatest when doses were administered on Day 11 of gestation. Zinc chloride, given at 12.5 mg/kg on day 11 of gestation, induced nonsignificant incidences of both skeletal and soft tissue defects compared to controls. No deaths were observed in the gravid females and no ripple ribs were observed in their fetuses.
Remark	:	Developmental toxicity data for several zinc compounds are available. Second-generation mice (from mothers fed zinc carbonate) exposed to high doses of zinc throughout the gestation, lactation, and postweaning periods had elevated levels of zinc in their bones, decreased blood copper levels, lowered hematocrit values and reduced body weights. The offspring of

pregnant rats fed zinc carbonate (500 mg Zn/kg) did not demonstrate any increase in the incidence of malformations. (WHO, 2001, Environmental Health Criteria 221, Zinc). Several developmental toxicity studies have been conducted with zinc sulfate on mice, rats, hamsters and rabbits, in general accordance with OECD Guideline 414; however, the form of the zinc sulfate was not specified. Depending upon the form that was used, the calculated NOAEL values ranged from 6.8 mg Zn/kg bw for the mouse to 35.2 mg Zn/kg bw for the hamster. (Risk Assessment for Zinc Metal, 2001, draft).

Reliability : 2 (reliable with restrictions): Comparable to guideline study with adequate documentation.

Reference : Chang, C-H., D.E. Mann, and R.F. Gautieri. 1977. Teratogenicity of zinc chloride, 1,10-phenanthroline, and a zinc-1,10-phenanthroline complex in mice. J. Pharm. Sci., 66:1755-1758.

5.8.3 TOXICITY TO REPRODUCTION

Type	:	Single-generation pilot breeding study
Guideline	:	Not specified
In vitro/in vivo	:	In vivo
Species	:	Rat
Strain	:	Sprague-Dawley SDTM
Sex	:	Both male and female
Route of admin.	:	Oral gavage
Exposure period	:	Males: Prior to cohabitation (77 d) and during cohabitation (21 d) Females: Prior to cohabitation (77 d), during cohabitation (21 d), and throughout gestation (21 d) and lactation (21 d).
Frequency of treatment	:	7 days/week
Duration of test	:	140 days (20 wk)
Doses	:	0, 7.5, 15, and 30 mg $ZnCl_2$/kg/d
Control group	:	Yes
Year	:	2001
GLP	:	No
Test substance	:	Zinc chloride
Method	:	Single generation breeding study
Method detail	:	Male and female rats (10 each per treatment) were administered 0.0, 7.5, 15.0, or 30.0 $ZnCl_2$ for 77 days prior to mating. At the end of the pre-mating period, males and females were paired within the same dose groups. Dosing was continued for both sexes throughout mating. All males were euthanized at the conclusion of mating, weighed, necropsied, and examined for morphological changes. Dosing was continued for females throughout gestation and lactation. Pregnant females were allowed to deliver their offspring naturally. Litter sizes were standardized on day 4 after birth to 4 of each sex. At day 21 of lactation, all F_0 females were sacrificed, necropsied, and examined for morphological changes. The evaluation of reproductive performance included fertility, viability index, weaning index, litter size, and the body weight of pups on days 0, 4, 7, 14, and 21 of lactation.
Results	:	The fertility indices in all dose groups were significantly lower than in the control group, but did not show a dose-response relationship. Pup viability indices on days 0 and 4 for the high-dose group were significantly lower than those of the control group. The body weights of pups in the highest dose group on days 14 and 21 were significantly lower than those in the control group. There were no effects on weaning indices or sex ratios. Overall, the results suggested that $ZnCl_2$ has only mild effects on rat reproductive performance up to 30 mg/kg/d. In addition, there were no significant treatment-related changes observed in any of the clinical

		pathology parameters that were evaluated. All histopathologic effects related to treatment were mild. Those in the reproductive organs were confined to males only and according to the authors probably precluded any adverse effects upon reproduction.
Remark	:	The effects on reproduction of other zinc compounds have also been studied. The LOAEL for serious reproductive effects in female rats was 200 and 250 mg Zn/kg/d from exposure to zinc sulfate and zinc carbonate, respectively, in the diet. (ATSDR, 2003, Draft Toxicological Profile for Zinc).
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Khan, A.T., A. Atkinson, T.C. Graham, M. Green, S. Ali, S.J. Thompson, and K.F. Shireen. 2001. Effects of low levels of zinc on reproductive performance of rats. Environ. Sci. (Tokyo), 8(4): 367-381.

Type	:	Sperm chromatin structure
Guideline	:	None
In vitro/in vivo	:	In vivo
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male
Route of admin.	:	Diet
Exposure period	:	8 weeks
Frequency of treatment	:	Continuous
Duration of test	:	8 weeks
Doses	:	4, 12, or 500 mg Zn/kg of diet (ppm)
Control group	:	No
Year	:	1993
GLP	:	No
Test substance	:	Zinc chloride
Method	:	
Method detail	:	Three-week old male rats (10 per group) were fed experimental diets with concentrations of zinc considered to be deficient (4 mg/kg), adequate (12 mg/kg) or excessive (500 mg/kg). After 8 weeks of feeding, animals were sacrificed to obtain testicular germ cells and epididymal sperm. Flow-cytometric procedures were used to determine effects on rat testicular development, including integrity of caudal epididymal sperm chromatin structure defined as the susceptibility of DNA to denaturation *in situ*.
Results	:	Rats fed the zinc deficient (4 ppm) diet demonstrated significant deviations in the ratio of testicular cell types present, including a reduction of S phase and total haploid cells. In addition, approximately 50% of epididymal sperm has a significant decrease in resistance to DNA denaturation *in situ*. Rats fed either a Zn-adequate or Zn-excess diet did not demonstrate an abnormal testicular cell type ratio. Excess Zn had a negative effect on chromatin structure, but much less than that of Zn deficiency.
Remark	:	Rats fed zinc chloride daily over an 8 week period demonstrated altered sperm chromatin structure with a LOAEL of 25 mg Zn/kg/d.
Reliability	:	2 (reliable with restrictions): Comparable to guideline study with adequate documentation.
Reference	:	Evenson, D.P., R.J. Emerick, L.K. Jost, H. Kayongo-Male, and S.R. Stewart. 1993. Zinc-silicon interactions influencing sperm chromatin integrity and testicular cell development in the rat as measured by flow cytometry. J. Anim. Sci., 71:955-962.

6.0 OTHER INFORMATION

6.1 Carcinogenicity

No adequate experimental evidence has been found to indicate that zinc salts administered orally or parenterally are tumorigenic. (WHO, 2001, Environmental Health Criteria 221, Zinc).